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The Journal of Laboratory and Clinical Medicine

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TABLE OF CONTENTS ON INSIDE COVER

The Journal of Laboratory and Clinical Medicine

CONTENTS FOR AUGUST, 1939

Clinical and Experimental

The Effect of Vitamin C on Lead Poisoning. Harry N. Holmes, Ph.D., Kathryn Campbell, Oberlin, Ohio, and Edward J. Amberg, M.D., Toledo, Ohio ---- 1119

A Comparison of Tests for Insulin Sensitivity. Paul H. Burgert, M.D., Walter H. Nadler, M.D., and Ruth Stott, A.B., Chicago, Ill. ---- 1128

An Unusual Case of Autoagglutination. K. M. Wheeler, Ph.D., H. J. Gallagher, M.D., and C. A. Stuart, Ph.D., Providence, R. I. ---- 1135

Gluconic Acid as a Urinary Acidifying Agent in Man. Harry Gold, M.D., and Helen Civin, M.S., New York, N. Y. With the Assistance of Charles Salzman, B.S. ---- 1139

Staphylococcus Epidermis Albus. Esther Meyer, Ph.G., M.S., Chicago, Ill. ---- 1146

A Study of Enzymes in Normal and Pathologic Cerebrospinal Fluid. I. Kaplan, Ph.D., D. J. Cohn, Ph.D., A. Levinson, M.D., and B. Stern, B.S., Chicago, Ill. ---- 1150

Normal Hematologic Standards in the Aged. Isidore Miller, M.D., Staten Island, N. Y. ---- 1172

Laboratory Methods

A Short Cut in Gas Analysis. F. S. Cotton, D.Sc., Sydney, Australia ---- 1177

Detection of Blood by Means of Chemiluminescence. Frederick Proescher, M.D., San Jose, Calif., and A. M. Moody, M.D., San Francisco, Calif. ---- 1183

A Modification of the Brown Anaerobe Jar. John H. Brewer, Ph.D., Baltimore, Md. ---- 1190

An Oxygenator With a Large Surface-Volume Ratio. John H. Gibbon, Jr., M.D., Philadelphia, Pa. ---- 1192

Determination of Sulfanilamide in Tissue, Urine, and Blood. Frank T. Maher, M.S., and W. J. R. Camp, M.D., Ph.D., Chicago, Ill. ---- 1198

The Optimum Dose of Sulfanilamide for Guinea Pigs. Arthur Riley Armstrong, B.S., M.D., and J. Lyndon Thompson, Ontario, Canada, With the Technical Assistance of Amelia Ruth Thomas, M.T. ---- 1203

A Micrometer Curve Measuring Apparatus. J. A. E. Eyster, M.D., and J. S. Hipple, Madison, Wis. ---- 1205

A Method of Isolating Larvae of Trichinella Spiralis for the Preparation of the Antigen Used for Immunologic Reactions in Trichinosis. H. Tsuchiya, Sc.D., St. Louis, Mo. ---- 1207

Free Chlorine as a Source of Error in Blood Sugar Determinations. Arthur K. Anderson, Ph.D., and Isadore Zipkin, B.A., State College, Pa. ---- 1209

A Method of Pancreatectomy in Pigeons. J. M. Janes, B.A., M.D., Toronto, Canada ---- 1210

An Electric Pulse Counter. Laurence E. Morehouse and W. W. Tuttle, Iowa City, Iowa ---- 1213

Department of Reviews and Abstracts

Selected Abstracts ---- 1217

Book Reviews ---- 1223

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The Journal of Laboratory and Clinical Medicine

VOL. 24 AUGUST, 1939 No. 11

CLINICAL AND EXPERIMENTAL

THE EFFECT OF VITAMIN C ON LEAD POISONING

HARRY N. HOLMES, PH.D., KATHRYN CAMPBELL, OBERLIN, OHIO, AND EDWARD J. AMBERG, M.D., TOLEDO, OHIO

IN a large industrial plant where the lead hazard was great due to fine metallic lead dust from filing down lead alloy seams, lead fumes from melting pots, and lead contacts from putty spray guns, there was an exceptional opportunity to diagnose and treat lead absorption and poisoning.

Four hundred men exposed to lead during their daily tenure at the plant were examined physically, and by laboratory methods. The latter consisted of monthly checks on the basophilic aggregation (B.A.), of the blood smear, according to McCord,[1] and on the degree of stippling, using Wright's stain. Records of these monthly tests were kept throughout the period of observation from April, 1937, until April 1, 1938. During the last three months of this period weekly tests were recorded on a cooperative group of 34, all of whom had symptoms and showed signs of lead poisoning. In addition to the above-mentioned tests, differentials were recorded on the Wright's smear, using the classification of Farley and others,[9] as used by Steinberg.* Urinalyses were not done due to technical difficulties at the factory.

During the period from April 1, 1937, to November 15, 1937, the gluconate, lactate, and diphosphate salts of calcium were given in the amount daily of from 80 to 100 grains to those who showed lead absorption by physical or laboratory signs. In addition, vitamin D (940 units) and vitamin A (9400 units) were given daily, using an accredited fish-liver oil.

The criterion used was a basophilic aggregation percentage of 1.5 or greater, abnormal stippling, or by request of the ill individual. Biweekly intravenous injections of 10 c.c. of 10 per cent calcium gluconate, together with daily oral therapy with calcium salts and vitamin D,[1] were given to

*Dr. Bernhard Steinberg, Director of Laboratories, Toledo Hospital.

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those whose basophilic aggregation registered higher (3.0 plus). Deleading with halides of potassium and ammonia were not done.

The period of intimate observation of the group of 34 lead poison patients upon whom we base this report began in November, 1937. It was then noted that, in spite of the intensive treatment with calcium and vitamin D, and the prophylactic measures taken by the plant management to reduce the lead hazard, many of the men failed by physical and laboratory standards to recuperate. In some, while there was a return to normal in percentage of basophilic aggregation and degree of stippling, there remained the complaint of ill-health, or abnormal cell morphology, or both.

Most frequent complaints were fatigue, anorexia, sleeplessness, or restless sleep; pains in the legs (gastrocnemius muscles) or in the forearms near the elbow; tenderness on deep pressure over the same areas, and irritability. Less frequently, complaint was made of trembling, constipation, sexual impotence, and joint pain. Observations on these patients consisted mainly of pyorrhea, spongy gums, and poor dentition in 75 per cent; tremor of varying degree, involving fingers and hand, usually bilaterally, and pallor of varying degree in 50 per cent; weakened extensibility at the wrist in about 10 per cent. One of the most striking signs was the surliness encountered in the majority of these subjects.

Ascorbic Acid Treatment.—One of us (E. J. A.) made the observation that the symptoms and signs exhibited by these men were similar to those found in subclinical scurvy. Ascorbic acid oral therapy was instituted in a group of 34 patients with chronic lead poisoning according to the following plan: Of 34 workmen (chronic patients) selected for this novel treatment, 17 were given ascorbic acid alone (at least two months after discontinuing the calcium salt injections), while an equal number continued the calcium therapy and at the same time took tablets of ascorbic acid.

Ascorbic Acid Without Calcium.—In general, the group of 17 patients made a marked gain in vigor, cheerfulness, color of skin, and blood picture. A week, or less than a week, after beginning the treatment of 100 mg. of ascorbic acid daily, most of the men enjoyed normal sleep, lost the irritability and nervousness so common with high calcium treatment of lead poisoning, enjoyed their food more, and no longer had tremors (if observed before). Several cases of leucopenia (probably due to a prolonged calcium therapy somewhat earlier) were cured by the ascorbic acid treatment.

Ascorbic Acid With Calcium.—The gain was less marked and rather irregular in the 17 patients given ascorbic acid with calcium. There was no gain in the number of mature neutrophiles in the blood—in strange contrast with the rapid gain when ascorbic acid alone was given.

In several instances alcohol nullified the good effects of calcium as well as of ascorbic acid, a complication that workmen exposed to lead hazards would do well to avoid.

CASE REPORTS

The detailed blood picture and the clinical observations for 3 workmen who received benefit from vitamin C in chronic lead absorption are outlined: The blood count was for 200 cells.

WORKMAN 1

CALCIUM GLUCONATE ALONE. LATER ASCORBIC ACID ALONE

DATE	B. A.* %	STIPPLING	RED BLOOD CELL MORPHOLOGY	MATURE NEUTROPHILES %	STABS %	JUVENILES %	MYELOCYTES %	LYMPHOCYTES %	EOSINOPHILES %
1937									
June	6	Strong							
Late June . . . Began biweekly intravenous injections of 10 c.c. 10 per cent calcium gluconate plus 30 grains calcium salts orally									
July	4.3	Strong							
Aug. 1	3.3	Strong							
. . . Discontinued calcium therapy. Plant closed Aug. 10 to Sept. 9. Lead hazard greatly reduced during September									
Oct.	3.0	Positive							
Nov.	2.1	Mild							
Feb.	1.4	Negative	Anisocytosis	24	2	0	2	68	4
Feb. 9 . . . Began daily doses of 100 mg. ascorbic acid									
Feb. 21	1.8	Strong		36	4	8	0	50	2
Feb. 28	1.5	Strong	Anisocytosis marked	49	0	2	0	55	2
Mar. 9	1.2	Mild	Normal	46	2	3	0	48	1
Mar. 16	1.0		Normal	66	2	4	0	18	6
Treatment discontinued									

*Basophilic aggregation.
Monocytes, none from Feb. 9 to March 16.
Basophiles, 0, 0, 1%, 0, 4% from Feb. 9 to March 16.

Clinically Workman 1 on February 9 showed definite symptoms of chronic lead poisoning with marked tremor, nervousness, irritability, sleeplessness. He was underweight, tired easily, and had a very sallow complexion. His blood picture was discouraging.

By March 16, after five weeks of ascorbic acid treatment, the tremor disappeared, and his complexion greatly improved. The man was very cheerful and no longer tired easily. The blood picture also greatly improved.

WORKMAN 2

CALCIUM SALT THERAPY. LATER ASCORBIC ACID ALONE

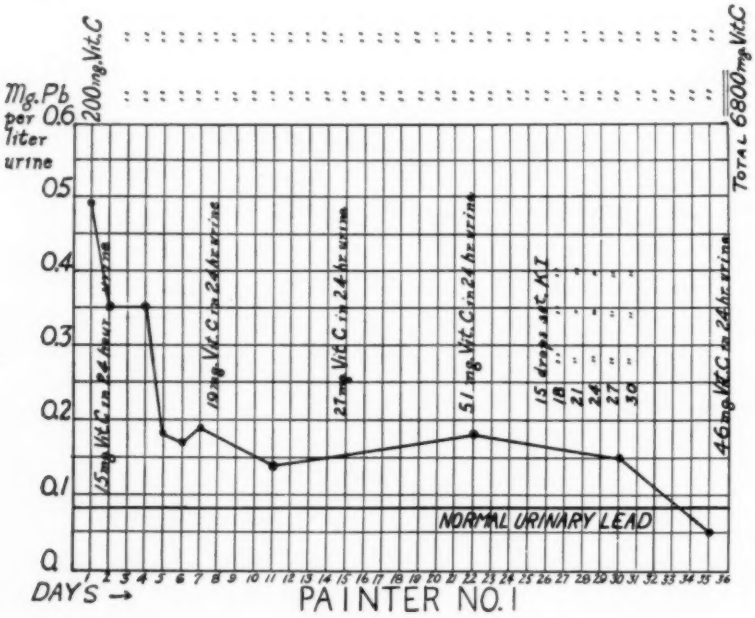
DATE	B. A.* %	STIPPLING	RED BLOOD CELL MORPHOLOGY	MATURE NEUTROPHILES %	STABS %	JUVENILES %	MYELOCYTES %	LYMPHOCYTES %	EOSINOPHILES %
1938									
Feb. 9	3.4	Positive	Anisocytosis moderate	38	0	6	2	48 (?)	0
Feb. 10 . . . Began biweekly intravenous injections of calcium gluconate. Calcium lactate and Natola orally daily									
Feb. 21	1.4	Negative	Achromia, anisocytosis slight	34	2	4	0	44	0
Feb. 24	0.9	Negative	Anisocytosis moderate	49	2	6	0	39	3
Mar. 3	3.1	Strong	Anisocytosis, polychromasia, achromia moderate	40	2	8	0	44	4
Mar. 7	1.2	Negative	Anisocytosis moderate	40	2	4	4	48 (?)	0
Mar. 7 Began daily doses of 100 mg. ascorbic acid. Calcium discontinued									
Mar. 14	1.3	Negative	Normal	62	2	0	0	34	1

*Basophiles, 0, 6%, 1%, 0, 1%, 0 for the six dates.
Monocytes, 6%, 10%, 0, 2%, 0, 1% for the six dates.

Clinically Workman 2 showed symptoms of chronic lead poisoning, with moderate tremor, nervousness, irritability, sleeplessness, and pallor. He tired easily. One week after beginning the ascorbic acid treatment, tremor and pallor were gone. He slept well, did not tire, and was extremely cheerful and talkative.

Workman 3 displayed clinically an acute case of lead poisoning in June, 1937, with a basophilic aggregation of 14, strong stippling, etc. Calcium therapy from June to February, 1938, lowered his basophilic aggregation satisfactorily, but three weeks after discontinuing this treatment, it increased considerably with return of the symptoms of acute lead poisoning.

He was then given 100 mg. of ascorbic acid daily for sixteen days and his basophilic aggregation was again lowered to normal (as with calcium therapy), with no clinical symptoms of lead poisoning. The morphology of the red blood cells became nearly normal. The man looked well and felt fine.



House Painters.—The research was transferred to Oberlin to make a study of ordinary house painters. Here it was possible to make analytical determinations of lead and ascorbic acid in the urine. The dithizone method for urinary lead, as described by Ross and Lucas,[2] and improved, especially in the use of a calculation curve, by Miller[3] and his associates, was used.

For the determination of ascorbic acid in urine the titration against the dye, 2.6-dichlorophenolindophenol, as described by Harris and Ray,[4] or Hawk,[5] was used. We found it necessary to devise improved methods of collecting twenty-four-hour specimens of urine in order to prevent the usual considerable losses of vitamin C (ascorbic acid) by oxidation. The details are given in another paper. Tests with a number of healthy people convinced us that the common estimate of an average daily urinary excretion of 15 mg. of vitamin C is too low, due to this oxidation loss, and that 25 mg. to 35 mg. is the probable average for healthy persons.

Of 14 painters observed clinically and by differential blood count, 2 were rated by clinical examination as chronic lead absorption patients, and the blood pictures added 5 more. Three of the 7 patients refused to cooperate further; hence we selected only 3 for study and treatment. Seven of the 14 picked up at random showed enough absorption to call for treatment or greater precautions.

CASE REPORTS

Case histories of 3 painters are here given. The final brief deleading was done by Dr. A. C. Siddall, who gave somewhat more potassium iodide than usual. In order to demonstrate more convincingly the value of a diet rich in vitamin C for men exposed to lead hazards, we determined the urinary lead and vitamin C of 2 more painters and gave them a brief deleading treatment. Furthermore, we determined the urinary vitamin C and lead of another painter who for many years had stressed fruits, vegetables, and milk in his diet.

Clinically the first painter and sprayer, who exhibited symptoms of chronic lead poisoning (physician's report) responded quickly to ascorbic acid treatment. After four days, he felt lazy and sleepy but not tired. Then he quickly changed to "feeling fine," ready for work, and fresh at the end of a hard day's work. At the same time there was a decided improvement in his blood picture. His urinary lead quickly fell to nearly normal, and his ascorbic acid excretion rose from 15 mg. per twenty-four hours to the very high value of 51 mg.

PAINTER 1

ASCORBIC ACID TREATMENT FOLLOWED BY DELEADING

DATE	B. A. %	STIPPLING	RED BLOOD CELL MORPHOLOGY	MATURE NEUTROPHILES %	STABS %	JUVENILES %	MYELOCYTES %	LYMPHOCYTES %	EOSINOPHILES %
1938									
May 11	0.9	7,500	Anisocytosis and slight polychromasia	32	9	8	0	43	5
July 7	1.0	6,000	Anisocytosis and slight polychromasia	30	10	6	0	45	6
July 7	. . .	Began 200 mg. ascorbic acid daily							
July 19	2.2	8,000	Anisocytosis moderate	50	10	4	0	31	3
Aug. 1	. . .	Continued ascorbic acid and began deleading with potassium iodide							
Aug. 7	. . .	End of deleading							
Aug. 9	1.6	Negative	Normal	62	2	1	0	30	2
Aug. 10	. . .	End of ascorbic acid treatment							

Basophiles, 2% May 11, 2% July 7, 2% July 19, 1% Aug. 9.
Monocytes, 1% May 11, 1% July 7, 0 July 19, 2% Aug. 9.

Clinically Painter 2 was rated by one of us (E. J. A.) as showing symptoms of chronic lead poisoning, even to a slight lead line on the gums. His teeth were bad and he tired quickly.

Two days of ascorbic acid treatment made him lazy and sleepy. After three weeks of ascorbic acid, he felt fine and energetic at the end of a hard day's work. His family noted the change from irritability to cheerfulness.

Urinary lead excretion fell from 0.4 mg. per liter to about 0.1 mg. Ascorbic acid in twenty-four hours' urine rose from 12 mg. to 86 mg.

Clinically Painter 3 seemed to one of us (E. J. A.) to be in good health, showing no signs of lead poisoning and having no complaints. During the period from May 9 to May 23 his blood picture was not reassuring and plans were made for ascorbic acid

PAINTER 2

ASCORBIC ACID TREATMENT CONTINUED THROUGH DELEADING

DATE	B. A. %	STIPPLING	RED BLOOD CELL MORPHOLOGY	MATURE NEUTROPHILES %	STABS %	JUVENILES %	MYELOCYTES %	LYMPHOCYTES %	EOSINOPHILES %
1938									
May 5	2.1	Negative	Anisocytosis slight	35	4	8	0	52 (?)	1
July 7	2.5	Negative	Anisocytosis moderate	28	6	10	0	54	2
July 7	. . .	Began 200 mg. ascorbic acid daily							
July 14	2.1	3,500	Anisocytosis moderate, poikilocytosis slight, polychromasia slight	40	15	4	1	36	2
July 28	1.4	Negative	Normal	64	2	1	0	28	3
July 29	. . .	Discontinued ascorbic acid. Deleading with potassium iodide began and continued three days until an attack of colic							
Aug. 9	2.3	3,000	Anisocytosis moderate, poikilocytosis slight, polychromasia slight	32	7	1	0	54	4

Basophiles, 2% Aug. 9. None on other dates.
Monocytes, 0 May 5, 0 July 7, 2% July 14, 2% July 28, 0 Aug. 9.

treatment. Urine examined for lead showed 0.5 mg. per liter (as compared to the average man's 0.085 mg.). Vitamin C excretion per twenty-four hours was very low, being only 10 mg.

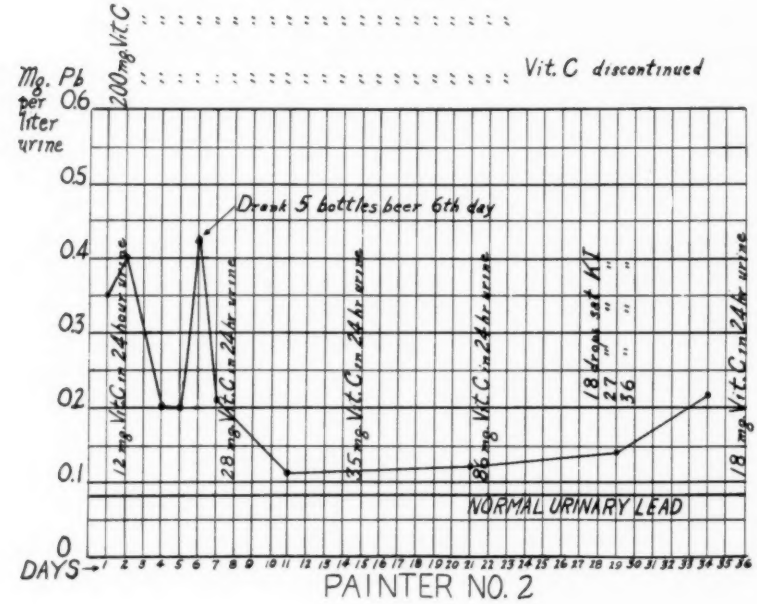
Two weeks of ascorbic acid treatment decidedly improved his blood picture and astonishingly lowered the urinary lead to 0.18 mg. per liter. Ascorbic acid excretion rose to the level of 31 mg. daily.

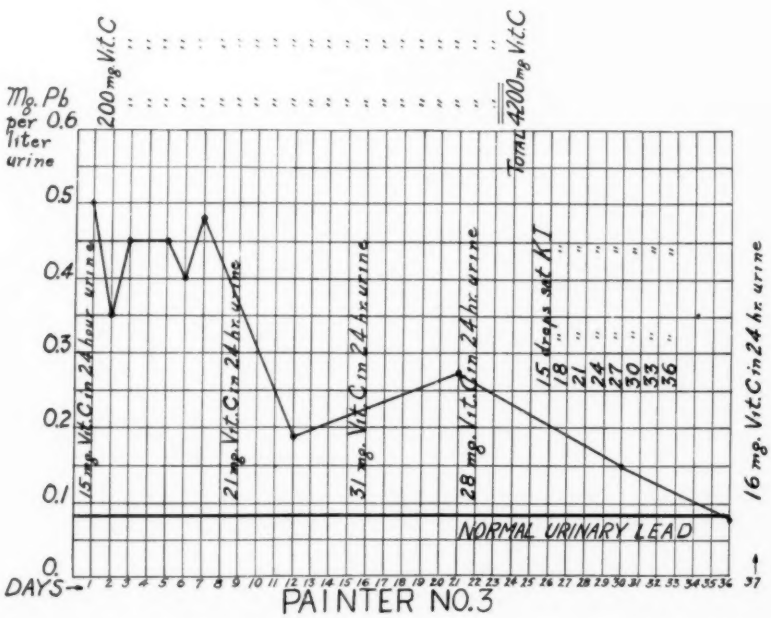
PAINTER 3

ASCORBIC ACID TREATMENT FOLLOWED BY DELEADING

DATE	B. A. %	STIPPLING	RED BLOOD CELL MORPHOLOGY	MATURE NEUTROPHILES %	STABS %	JUVENILES %	MYELOCYTES %	LYMPHOCYTES %	EOSINOPHILES %
1938									
May 9	1.8	30,000	Anisocytosis and polychromasia moderate	44	13	2	1	26	10
May 23	3.2			34	24	2	0	31	7
July 7	2.0	15,000	Anisocytosis moderate, poikilocytosis slight	30	12	3	0	47	6
July 7	. . .	Began 200 mg. ascorbic acid daily							
July 21	1.6	Negative	Normal	58	2	2	0	34	3
July 29	. . .	Discontinued ascorbic acid. Began deleading with potassium iodide (15 drops saturated solution, increasing by 3 drops daily)							
Aug. 8	1.0	Negative	Normal	57	4	1	0	35	3

Basophiles, 2% May 9, 2% May 23, 2% July 7, 1% July 21.
Monocytes, 2% May 9. None on other dates.


200 mg Vit C
Vit. C discontinued
Mg. Pb per 0.6 liter urine
0.5
0.4
0.3
0.2
0.1
0
Drank 5 bottles beer 6th day
12 mg. Vit C in 24 hour urine
28 mg. Vit. C in 24 hr. urine
35 mg. Vit. C in 24 hr. urine
86 mg. Vit. C in 24 hr. urine
18 drops sat. KI
27 " "
36 " "
18 mg. Vit. C in 24 hr. urine
NORMAL URINARY LEAD
DAYS → 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
PAINTER NO. 2


200 mg Vit. C
Total 4200 mg Vit. C
Mg. Pb per 0.6 liter urine
0.5
0.4
0.3
0.2
0.1
0
15 mg. Vit. C in 24 hour urine
21 mg. Vit. C in 24 hr. urine
31 mg. Vit. C in 24 hr. urine
28 mg. Vit. C in 24 hr. urine
15 drops sat. KI
18 " "
21 " "
24 " "
27 " "
30 " "
33 " "
36 " "
16 mg. Vit. C in 24 hr. urine
NORMAL URINARY LEAD
DAYS → 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
PAINTER NO. 3

Eight days of deleading (ascorbic acid discontinued) brought urinary lead to normal (0.085 mg. per liter), but the ascorbic acid excretion unfortunately fell to 16 mg. per twenty-four hours. Clinically the man seemed in good health throughout the deleading treatment.

Painter 4.—Painter 4, a house painter for twenty-five years, showed the effects of a diet unusually rich in the best vitamin C foods as well as in milk. His health (except for a temporary tooth infection) was good, his urinary lead was only 0.1 mg. per liter, and his ascorbic excretion was 58 mg. per twenty-four hours, an exceptional figure.

Deleading with 80 grains ammonium chloride daily for six days was done (no milk in that period) and the urinary lead was cut to 0.04 mg. per liter.

Painter 5.—The son of Painter 4, Painter 5 was brought up in the same dietary tradition as his father. He was in excellent health and painted for several years. He excreted 0.16 mg. lead per liter of urine. After six days of deleading with ammonium chloride (80 grains daily), his urinary lead excretion fell to 0.05 mg. He was comfortable during deleading. The usual vitamin C excretion had been 40 mg. per twenty-four hours urine.

Painter 6.—Painter 6 was a house painter who for fifteen years faithfully applied the dietary teachings of a religious organization and lived on a diet exceptionally rich in fruits and vegetables (many raw) and milk. He was in excellent health and possessed a good blood picture and a very good vitamin C excretion of 37 mg. per twenty-four hours. His lead excretion was 0.215 mg. per liter after four days of deleading with potassium iodide.

His wife on the same religious diet excreted 50 mg. of vitamin C per twenty-four hours, but in her case the effect of lead on vitamin C was not a factor. His pastor, a painter for thirty years, also living on a diet exceptionally rich in fruits, vegetables, and milk, enjoyed splendid health, and excreted only 0.13 mg. lead per liter in spite of his painting.

DISCUSSION

Administration of 100 mg. of vitamin C daily to each of 34 workmen exposed to factory lead hazards (and diagnosed as suffering from lead absorption) in general decidedly improved their blood picture and their health. Symptoms characteristic of chronic lead absorption usually disappeared. It seems reasonable to suppose that toxic lead ions were removed to a considerable extent from the blood stream. Calcium gluconate or lactate does this by storing the lead in the bones, presumably as lead phosphate. Experience shows some drawbacks to long-continued high calcium medication; hence bone storage should be followed by cautious deleading, with a respite from lead absorption.

A more detailed study of 3 house painters, diagnosed as suffering from chronic lead absorption, strongly reinforced the conclusions drawn from the factory experiments. The observation that 200 mg. daily of vitamin C as a dietary supplement decidedly lowered the urinary excretion of lead is astonishing. With it goes another observation that men actually suffering from lead absorption excrete less vitamin C in the urine than does the average man.

It is difficult to escape the conclusion that toxic lead compounds react with vitamin C to form a poorly ionized salt (lead ascorbate?) or a complex salt which yields a very low concentration of simple lead ions. If solutions of lead acetate and vitamin C are mixed in a test tube, no precipitate is observed; hence the product is soluble, not to be precipitated in the bones. Cautious addition of sodium hydroxide fails to precipitate lead hydroxide or oxide, normally termed insoluble.

This view of removal of toxic lead ions by reaction with ascorbic acid to form soluble, but poorly ionized, salts or complexes is strengthened by the observation of Greenwald[6] on the remarkable influence of ascorbic acid on the dissociation (and solubility products) of calcium phosphate and certain other calcium salts. He is of the opinion also that calcium gluconate, lactate, and citrate form complexes with calcium ions. Hastings and McLean[7] offer a parallel in the fact that citrates lower the concentration of free calcium ions through formation of a soluble complex.

In an excess of enthusiasm over the good diet, one of our painters drank a pint of milk for five days before starting the ascorbic acid treatment.

His vitamin C excretion, previously only 10 to 13 mg., quickly rose to 25 mg. and, after he discontinued his daily pint of milk, quickly fell to 15 mg. A similar observation was made with another painter. Evidently sufficient dietary calcium spares vitamin C in the body of a man suffering from lead absorption, probably by forcing some of the lead into the bones as lead phosphate.

A natural fear arises that if ascorbic acid therapy reduces urinary excretion of lead this lead must be stored somewhere in the body, to be released later in toxic form in the blood stream as dietary or other conditions change. This was not true of four or five local house painters who enjoyed several, even many, years of excellent health while painting, evidently because of a diet that we know to be extremely rich in vitamin C. At any rate, we found that they excreted more of this vitamin than does the average healthy individual.

Judging from the statement by Sollman on deleading with potassium iodide, it seems a fair assumption that the compound formed by ascorbic acid and toxic lead ions is preferentially absorbed by the liver and, with the aid of the bile, excreted in the feces. We hope that others interested in this research will determine this by quantitative analysis. He[8] writes: "Renal excretion of lead depends directly upon the lead compounds circulating at the time in true solution in the blood. . . . The increased excretion (of lead) through use of potassium iodide has been noted by previous workers, although others have failed to confirm it, chiefly because they confined themselves to the urine while the excretion is mainly in the faeces. The iodide causes the reappearance of lead in the faeces from which it had been absent."

Note.—An extra 50 mg. more or less of vitamin C is furnished by approximately

4 oz. fresh orange juice
4 oz. grapefruit
4 oz. raw cabbage
2 oz. raw spinach (salad)
4 oz. spinach, cooked in least possible water
1 oz. raw green pepper (salad)
8 oz. tomatoes, whole or juice
8 oz. cantaloupe
4 oz. raw turnips

In general, fresh fruits and vegetables (and canned tomatoes) are very good sources of vitamin C, but the average workman will find the above list definite and practical. On a limited income even raw cabbage and turnips with canned tomatoes are possible as sources of 50 mg. or 100 mg. vitamin C daily.

REFERENCES

1. McCord, C. P., and Johnson, J.: The Basophilic Aggregation Test, Am. J. Pub. Health **25**: 1089, 1935.
2. Ross, J. R., and Lucas, C. C.: A New Method for the Determination of Minute Amounts of Lead in Urine, J. Biol. Chem. **3**: 290, 1935.
3. Winter, O. B., Robinson, H. M., Lamb, F. W., and Miller, E. J.: The Determination of Lead, Indust. & Engin. Chem. Analyt. Ed. **7**: 265, 1935.
4. Harris, L. J., and Ray, S. N.: Diagnosis of Vitamin C in Subnutrition by Urine Analysis, Lancet **1**: 71, 1935.
5. Hawk, P. B., and Bergeim, O.: Practical Physiological Chemistry, ed. 11, Philadelphia, 1937, P. Blakiston's Son & Co., p. 733.
6. Greenwald, I.: The Dissociation of Some Calcium Salts, J. Biol. Chem. **124**: 437, 1938.
7. McLean, F. C., and Hastings, A. B.: A Biological Method for the Estimation of Calcium Ion, J. Biol. Chem. **107**: 351, 1934.
8. Sollman, T.: A Manual of Pharmacology, ed. 3, Philadelphia, 1928, W. B. Saunders Co., p. 1049.
9. Farley, D. L., St. Clair, H., and Reisinger, J. A.: Am. J. M. Sc. **180**: 336, 1930.

A COMPARISON OF TESTS FOR INSULIN SENSITIVITY*

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THE use of insulin early disclosed variations in response. Radoslav[1] in 1924 studied the effect of 20 units subcutaneously in 29 fasting patients with diabetes as well as in normal individuals and patients with renal glycosuria, and proposed this test for differential diagnosis. The fall in blood sugar ranged between 34 and 77 per cent, and was most marked when the original blood sugar was high. Intravenous insulin, 10 units, was given in several cases as a check. One patient with diabetes exhibited resistance to insulin; 20 units subcutaneously and 10 and 20 units intravenously produced little effect even though the original level was high. Falta and his associates continued these studies and in his monograph published in 1936, Falta[2] based his discussion on the classification of diabetic patients as sensitive and resistant to insulin. In 362 patients, 34.2 per cent were sensitive, 51.4 per cent were resistant, and 14.4 per cent were intermediary, but resembled the resistant persons. Obesity was more than twice as frequent in resistant and intermediate as in sensitive patients.

MacBryde[3] in 1936 reported similar tests to be of value as an index to dietary management. Fifteen patients fell into one of two groups: insulin sensitive patients failed to gain tolerance with a higher carbohydrate diet, while relatively resistant cases gained tolerance when the carbohydrate allowance was increased. Himsworth[4] in 1936, using an insulin-glucose test and examining both arterial and venous blood sugar samples, also obtained two distinct types of curves, but reported contrary conclusions. Classified according to his method, insulin sensitive patients were able to utilize large increases in carbohydrate without additional insulin, whereas insensitive persons were unable to do so. The contradiction in results reported by these writers has, as stated by Wilder[5] who has reviewed the subject in detail, only added to the existing confusion.

Our experience with the Himsworth test in 9 patients[6] had revealed striking differences between typical curves of two types and had suggested that intermediate curves might be obtained. In general, the clinical conclusions of Himsworth as to the effect of dietary changes were supported. In the present study 14 patients who had been classified as to insulin sensitivity by the Himsworth method were tested by the intravenous insulin method of MacBryde. Subsequently 13 of these patients were examined by the subcutaneous insulin method.

PROCEDURE

The test described by Himsworth was used with only slight modification. Capillary and venous blood samples were drawn from fasting patients believed to be stabilized after months or years of treatment. Regular insulin, 5 units per square meter of body surface, as determined from a nomogram, was then in-

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jected intravenously; immediately afterwards 30 gm. of dextrose per square meter of body surface were given by mouth. Venous and capillary blood samples were obtained at intervals of five, ten, twenty, thirty, forty, fifty, and sixty minutes after ingestion of dextrose.

The insulin tests of Radoslav were performed as described by MacBryde. With the subcutaneous method, specimens of capillary blood were examined hourly for four hours after the injection into the fasting patient of 1 unit of insulin per 10 pounds of body weight. The per cent of fall in blood sugar from the fasting level to the lowest point observed was computed. With the intravenous method, $\frac{1}{15}$ unit of insulin per kilogram body weight was given in the fasting state, and capillary blood samples were drawn every fifteen minutes for one and one-half hours.

Blood sugar determinations were made by means of a modification of the Folin-Wu micromethod.[7] Duplicate tests were made in nearly all instances.

TABLE I

PATIENT	SEX	AGE	WEIGHT PER CENT NORMAL	DURATION	CARBOHYDRATE ALLOWANCE (GRAMS)	INSULIN	ASSOCIATED FINDINGS
1 (M.G.)	M	18	- 7	8 years	160	40P+10	
2 (B.A.)	M	16	Normal	6½ years	150	40P+10	
3 (H.K.)	F	26	-10	7½ years	150	40P+10	
4 (H.W.)	M	29	-20	9 months	215	15P	
5 (B.B.)	F	52	+10	4 years	138	20P	
6 (H.H.)	M	18	+20	3 years	150	40P+20	
7 (F.S.)	F	14	Normal	5 years	183	40P+20	
8 (T.S.)	M	33	- 6	8 years	110	10P	
9 (M.R.)	F	35	-25	7 years	165	10P	Constitutional inferiority
10 (G.)	F	34	- 5	5 years	165	16P	Hay fever
11 (W.)	F	39	- 5	1½ years	150	35P	
12 (A.G.)	F	40	- 5	14 years	160	50P+12	
13 (F.M.)	F	57	-15	5 years	160	40P	Hypertension; hay fever
14 (H.S.)	M	57	Normal	8 years	110	10P	Arteriosclerosis; angina

RESULTS

Clinical data concerning the patients studied appear in Table I. Their ages ranged from 16 to 57 years. In all patients except 2 the diabetes had been treated for at least three years. Only 2 patients were overweight; 6 were underweight within 10 per cent, and 3 over 15 per cent. Definite clinical arteriosclerosis was present in only 2 cases. Two patients gave a history of recurrent hay fever. Patient 7 was a daughter of Patient 8. The carbohydrate allowances ranged from 110 to 215 gm. and averaged 154 gm. The diets were adequate in protein content and in calories. All the patients had been treated with protamine zinc insulin for at least six months.

Classification according to the insulin-glucose method of Himsworth and to the subcutaneous and intravenous insulin tests of MacBryde is listed in Table II.

The Himsworth test showed striking differences between insulin sensitivity and insensitivity in the majority of cases. In several instances the arterial and venous blood sugar curves were not typical in every respect, but they showed general characteristics either of sensitivity or insensitivity. *In 14 patients the Himsworth method showed 5 sensitive and 9 insensitive.*

TABLE II

PATIENT	HIMSWORTH		MACBRYDE: SUBCUTANEOUS				MACBRYDE: INTRAVENOUS			
		BLOOD SUGAR BEFORE TEST (MG. %)		FASTING BLOOD SUGAR (MG. %)	LOWEST BLOOD SUGAR	PER CENT FALL		FASTING BLOOD SUGAR (MG. %)	LOWEST BLOOD SUGAR	PER CENT FALL
1	Sensitive	–	Sensitive	119.0	25.0	79.0	Sensitive	113.0	30.0	73.0
2	Sensitive	150	Sensitive	338.5	49.5	85.0	*Borderline*	69.4	52.9	24.0
3	Sensitive	206	Sensitive	264.0	77.0	71.0	Sensitive	94.4	30.7	66.0
4	Sensitive	173	Sensitive	156.4	46.6	70.8	Sensitive	105.2	34.4	67.0
5	Sensitive	143	*Insensitive*	212.2	113.4	47.0	Sensitive	196.6	92.6	53.0
6	Insensitive	126	*Sensitive*	214.4	40.0	81.0	*Borderline*	250.0	186.9	25.0
7	Insensitive	169	*Borderline*	249.0	114.2	54.0	Sensitive	84.5	42.8	49.0
8	Insensitive	77	–	–	–	–	Sensitive	46.2	15.2	67.0
9	Insensitive	60	Insensitive	75.5	59.9	20.0	Sensitive	74.0	43.5	41.0
10	Insensitive	90	Insensitive	105.2	50.2	52.0	Sensitive	107.5	53.0	51.0
11	Insensitive	126	*Sensitive*	229.5	79.4	65.0	Sensitive	113.0	49.5	56.0
12	Insensitive	54	*Sensitive*	61.0	23.7	61.0	Sensitive	197.0	47.8	75.0
13	Insensitive	140	Insensitive	171.5	97.4	43.0	*Borderline*	107.0	72.8	32.0
14	Insensitive	116	Insensitive	143.0	119.0	16.8	Insensitive	148.5	130.5	8.2

Classification according to the insulin tolerance tests (columns 2 and 3) is based on the percentage of fall in blood sugar found by MacBryde and reported in 15 cases. (In cases of insulin sensitivity he observed a fall of 61 to 75 per cent by the subcutaneous and of 34 to 45 per cent by the intravenous method. In relatively resistant cases a fall of 32 to 53 per cent was found with the subcutaneous and of 12 to 22 per cent with the intravenous test. Percentages lying between these ranges are called border line.)

With the insulin tolerance tests used by MacBryde, interpretation and classification were often difficult because of the lack of a definite point of demarcation between the percentages of fall in blood sugar of sensitive and relatively resistant cases. The ranges of percentage fall in blood sugar reported by MacBryde in 15 cases were arbitrarily chosen as a standard of differentiation. Percentages lying between these levels were classed as border line. In our series the intravenous method tended to demonstrate sensitivity to insulin in most cases. In the 14 patients tested there was only one patient (No. 14) who was insulin resistant. This patient showed a much greater degree of resistance than any other reported by MacBryde and evidently was similar to the one patient found by Radoslav. *In the 14 patients classified according to the intravenous MacBryde method, 10 were insulin sensitive, 3 were border line, and one was insensitive. Two of the borderline patients verged toward sensitivity and one toward insulin resistance.* The definitely resistant case was also resistant with the other methods. One Himsworth sensitive person (No. 2) showed, with the intravenous MacBryde test, a drop in blood sugar level only slightly above the range reported by MacBryde in 3 resistant patients. *Of 9 Himsworth insensitive patients, sensitivity to insulin was indicated in 6 by the intravenous MacBryde method.*

The subcutaneous insulin tolerance test was compared with the Himsworth test in 13 patients. *With the subcutaneous MacBryde method, 7 patients were sensitive, 5 were insensitive or "relatively resistant," and one was border line verging on resistance. Of the 5 Himsworth sensitive patients, 4 were sensitive according to the subcutaneous MacBryde method, while one was insensitive. Of 8 Himsworth insensitive persons, 4 were likewise insensitive by means of the subcutaneous MacBryde test; one borderline patient was relatively resistant; 3 patients were sensitive.*

A comparison of the results of intravenous and subcutaneous insulin tolerance tests was made in 13 patients. As previously indicated the former method showed 9 sensitive, 3 border line, and one very resistant, while the latter showed 7 sensitive, 5 insensitive, and one border line. *Of the 9 patients sensitive to insulin by the intravenous method, only 5 showed comparable sensitivity to the subcutaneous test. Three patients insensitive according to the subcutaneous test were definitely sensitive with the intravenous method; one patient was insensitive with the subcutaneous and border line, verging on resistance, with the intravenous test.*

In general, although all three tests usually indicated sensitivity in patients who had experienced many insulin reactions, there were exceptions to this rule, notably Patient 12. This patient had a severe, unstable diabetes of 14 years' duration; insulin reactions were frequent. The Himsworth test disclosed insensitivity to insulin, while the MacBryde tests indicated sensitivity. One patient, No. 14, exhibited undoubted resistance to insulin by all tests. Disagreement with the Himsworth test by the other tests was most frequent in cases of Himsworth insensitiveness. While disagreement between the two MacBryde tests seemed due mainly to uncertainty as to the degree of relative resistance, other differences are not so readily explained. For example, Patient 9, an undernourished woman

of 35 years with constitutional inferiority, was insulin insensitive both by the Himsworth and subcutaneous MacBryde methods but definitely sensitive with the intravenous MacBryde test.

DISCUSSION

The following factors might influence the results obtained: the fact that the tests were made on ambulatory patients; the general condition at the time of the test; the time intervals between examinations; the carbohydrate content of the preceding diet; the use of protamine zinc insulin, including an injection twenty-four to twenty-six hours before each test; and the level of the fasting blood sugar.

Most of the patients had previous hospital education. Although in several patients the diabetes was unstable, in all of them the disease had for months been under good, or fairly good, control. They were apparently well and without complaints at the time of the tests. During the weeks or months between tests, the diet and insulin dosage were practically unchanged. The carbohydrate content of the diet, within the range of 110 and 215 gm., employed in this group of patients, was apparently not a factor. Patient 7, a juvenile with diabetes, receiving 183 gm. of carbohydrate, was Himsworth insensitive as was her father; the subcutaneous MacBryde test gave a borderline response, while the intravenous method indicated sensitivity to insulin.

The use of protamine zinc insulin up to twenty-four hours preceding the tests may undoubtedly have influenced the results, predisposing to sensitivity, in a number of cases. This factor was studied in three patients. In Patient 1, sensitive to all three tests, a fasting blood sugar of 67 mg. per 100 c.c. fell 53.8 per cent to 31 mg. after ninety minutes of continued fasting. Patient 4, however, also sensitive to all three tests, had a fasting blood sugar level of 99 mg. per 100 c.c., and one hour and thirty minutes later a fasting level of 120 mg. Patient 12, Himsworth insensitive and MacBryde sensitive, showed a drop of only 12 per cent (from 220 to 193 mg. per 100 c.c.) after one hour and thirty minutes of continued fasting. It seems evident that the use of protamine zinc insulin prior to examinations could not be entirely responsible for the conflicting results of the various tests used. In particular it is difficult to explain how a blood-sugar lowering effect, possibly prolonged for more than twenty-four hours, could be a factor in promoting the insulin-resistant responses.

The fasting blood sugar level is undoubtedly an important, if not the determining, factor in influencing the percentage of fall following the injections of insulin. This was clearly recognized by Radoslav. The high percentage of drop in blood sugar noted in Patients 2, 3, and 6, after the subcutaneous injection of insulin, is probably explained on this basis. In Patient 6, however, the intravenous MacBryde test revealed no comparable fall even though the fasting blood sugar was high.

Holcomb and Holcomb[8] have used the MacBryde test as a basis of classification in 7 patients. Six patients with high fasting blood sugars showed a precipitous drop; on computing the percentages of fall the variations ranged

from 45 to 75 per cent, with 3 under 55 per cent. The one patient considered insulin "tolerant," or relatively resistant, showed a drop of 60 per cent (from about 160 to 60 mg. per 100 c.c.). It is impossible to accept this case as one of "resistance" to insulin.

It seems plain from our data, and it is recognized by Falta, that borderline cases occur when insulin tolerance tests are used. Klatskin[9] applied the subcutaneous MacBryde test to 50 diabetic patients and found wide variations in the percentage fall of blood sugar, ranging from 30 to 85 per cent. Using a drop of 60 per cent as a dividing line, no natural cleavage between insulin sensitivity and resistance could be made out, and no significant relationship between sensitivity and response to high carbohydrate diets could be demonstrated. Soskin and Levine[10] have reported the reproduction in the same completely depancreatized dog, at different times, of both types of diabetes. Hepatic injury reversed the sensitive type of curve. They conclude that the significant difference between insulin sensitivity and relative resistance depends upon the functional capacity of the liver.

The Himsworth test has demonstrated insensitive types of curves in unusual cases of diabetes. One of our patients with acromegaly and diabetes (not included in this series) showed, before treatment of the diabetes, an intermediate curve with the general characteristics of insensitivity; three weeks later, after treatment with diet and insulin and after irradiation, more typical insensitive curves were obtained. Flaum[11] reported that insensitive types of curves were obtained in the study of diabetes associated with acromegaly and suggested that the phenomenon of Himsworth insulin-insensitivity when seen in diabetes uncomplicated by obvious endocrine dysfunction may possibly be due to hyperactivity of one or more of the endocrine glands. Marble[12] reported Himsworth insensitiveness in a diabetic requiring very large amounts of insulin without demonstrable explanation of this peculiarity. His patient had insulin reactions. Patient 12 in our series, although insulin insensitive, is an unstable diabetic person who has frequent reactions. According to Himsworth, the response to the test is not dependent upon liver function. The question arises whether this test is also reversible. Patient 12 was unmistakably insensitive on December 2, 1937. About ten months later the response was intermediate in that the arteriovenous differences had widened considerably; the curves were, however, still of the insensitive type.

de Wesselow and Griffiths,[13] instead of the Himsworth test, gave to a series of normal and diabetic patients 5 units of insulin intravenously and 50 gm. of glucose by mouth, and tested capillary blood sugar in the fasting state and after thirty and sixty minutes. Results were expressed as the algebraic sum of the differences between the initial blood sugar and the values thirty and sixty minutes after the insulin injection. No evidence of the existence of two distinct types of diabetes was obtained. Sensitivity to the test depended to a large extent upon an adequate carbohydrate allowance in the diet. It was found by this test that resistant diabetic patients may become sensitive when put on a high carbohydrate ration, adequately controlled by insulin.

CONCLUSIONS

1. Comparisons of classification as to insulin sensitivity or insensitivity between the Himsworth and the subcutaneous and intravenous MacBryde tests revealed no dependable correlation. In some patients all of the tests demonstrated definite evidence of marked insulin effect. Only one patient was unmistakably insensitive by all three methods. The subcutaneous and intravenous MacBryde tests did not agree in all instances.

2. The Himsworth and the insulin tolerance tests evidently do not give the same information. The former presents more striking and definite differences between insulin sensitivity and insensitivity. The latter methods present difficulties in appraising degrees of relative resistance; borderline cases occur. With the subcutaneous MacBryde test the greatest percentage fall in blood sugar usually occurs when the fasting level is high.

3. Classification as to insulin sensitivity or insensitivity should be made only in terms of the particular test used. The possible reversibility of the various tests is discussed. From the evidence available there appears to be no justification for the classification of diabetes as of two distinct types based solely on sensitivity or resistance to insulin.

REFERENCES

1. Radoslav, C. S.: Ueber die Wirkung des Insulins auf den Blutzucker beim Menschen, Wien. Arch. f. inn. Med. **8**: 395, 1924.
2. Falta, W.: Die Zuckerkrankheit, Berlin, Vienna, 1936, Urban & Schwarzenberg.
3. MacBryde, C. M.: Response to Insulin as an Index to the Dietary Management of Diabetes, J. Clin. Investigation **15**: 577, 1936.
4. Himsworth, H. P.: Diabetes Mellitus: Its Differentiation Into Insulin-Sensitive and Insulin-Insensitive Types, Lancet **1**: 127, 1936.
5. Wilder, R. M., and Wilbur, D. L.: Diseases of Metabolism and Nutrition: Review of Certain Recent Contributions, Arch. Int. Med. **59**: 329, 1937.
6. Nadler, W. H.: Sensitiveness to Insulin and Optimal Diets in Diabetes, Arch. Path. **26**: 395, 1938.
7. Nadler, W. H., Starr, P., and Tukey, G.: Glycemia as a Guide in the Treatment of Diabetes Mellitus: The Practicability of Routine Examinations of Small Effectively Preserved Specimens of Blood Drawn by the Patient, Arch. Int. Med. **36**: 579, 1926.
8. Holcomb, B., and Holcomb, R.: Response of Insulin-Sensitive and Insulin-Tolerant Patients to Protamine Insulin, Northwest Med. **37**: 36, 1938.
9. Klatskin, G.: The Response of Diabetics to a Standard Test Dose of Insulin, J. Clin. Investigation **17**: 745, 1938.
10. Soskin, S., and Levine, R.: Experimental Production of Insulin-Sensitive and Insulin-Insensitive Types of Diabetes, Abst., J. A. M. A. **110**: 768, 1938.
11. Flaum, G.: Insulin Sensitivity in Endocrine Disorders, Abst., Am. J. Physiol. **123**: 67, 1938.
12. Marble, A.: Insulin Resistance: Report of a Case of Marked Insensitiveness of Long Duration Without Demonstrable Cause, Arch. Int. Med. **62**: 432, 1938.
13. de Wesselow, O. L. V., and Griffiths, W. J.: Insulin Resistance in Diabetes Mellitus and the Effect of Dietary Carbohydrate, Quart. J. Med. **7**: 17, 1938.

AN UNUSUAL CASE OF AUTOAGGLUTINATION*

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COLD agglutinins or autoagglutinins occur normally in many human sera.[1] In various pathologic conditions, however, the titer may be greatly increased[2] and may interfere with the usual O, A, B, and AB blood grouping tests.[3] We encountered such a case in a routine typing for transfusion and have studied it in some detail.

The patient, G. C., a white female 38 years old, was admitted to the hospital December 1, 1937, complaining of a severe cough, weakness, and fever. She had been ill for five days with symptoms of upper respiratory infection, headache, and malaise which became progressively worse. On admittance the patient had a temperature of 102.4° F., a respiration and pulse rate of 25 and 100, respectively, and showed typical consolidation signs in the right lower lobe which were confirmed by x-ray. A pericardial friction rub and general toxemia with cyanosis were also noted. The white blood count was 14,150 with marked polymorphonuclear shift to left. The red blood count, hemoglobin, blood chemistry, and urine were normal. For thirteen days the temperature showed high septic spikes with corresponding increases in pulse and respiration. A pneumococcus which could not be typed was twice found in the sputum but blood cultures were consistently negative. Oxygen, high fluid, and symptomatic therapy were given. The general toxemia increased, with delirium and marked prostration. On December 10 an x-ray examination showed an increased mottled density in both lower lung fields with no fluid, and an electrocardiogram revealed suggestive pericarditis and myocarditis and myocardial involvement. A transfusion of 300 c.c. was given December 14 which was followed by a four-day drop of the vital signs (temperature, respiration, and pulse). A new rise in the vital signs occurred on Dec. 21 with constant pneumonitis in the right and left lower lung fields with a diagnosis of unresolved pneumonia and possible encapsulated empyema which yielded no fluid when the area was tapped. A second transfusion of 300 c.c. was given on December 27 without significant change in the course of the infection. By January 15 the vital signs had dropped to normal with a marked gain in the patient's general condition and clearing of the lungs. The remainder of the patient's course in the hospital was uneventful, except for occasional temperature spikes and associated arthralgia. The patient was discharged March 13 much improved in strength and general condition.

The first attempt at typing was made at room temperature using the patient's unwashed erythrocytes. Agglutination occurred in both the anti-A and anti-B sera as well as in the saline control. When the cells were washed twice

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with saline, however, they agglutinated only in anti-A serum and were subsequently subgrouped as A_1 type MN. The patient's serum agglutinated O, A_1, and autologous cells at room temperature to a titer of 320. The reactions were completely reversible by warming to 37° C. A group A (A_1) donor was selected whose cells were not agglutinated at 37°, but who reacted strongly with the patient's serum at 22°, and a successful transfusion of 300 c.c. was performed. Two weeks later a second transfusion from the same donor was given.

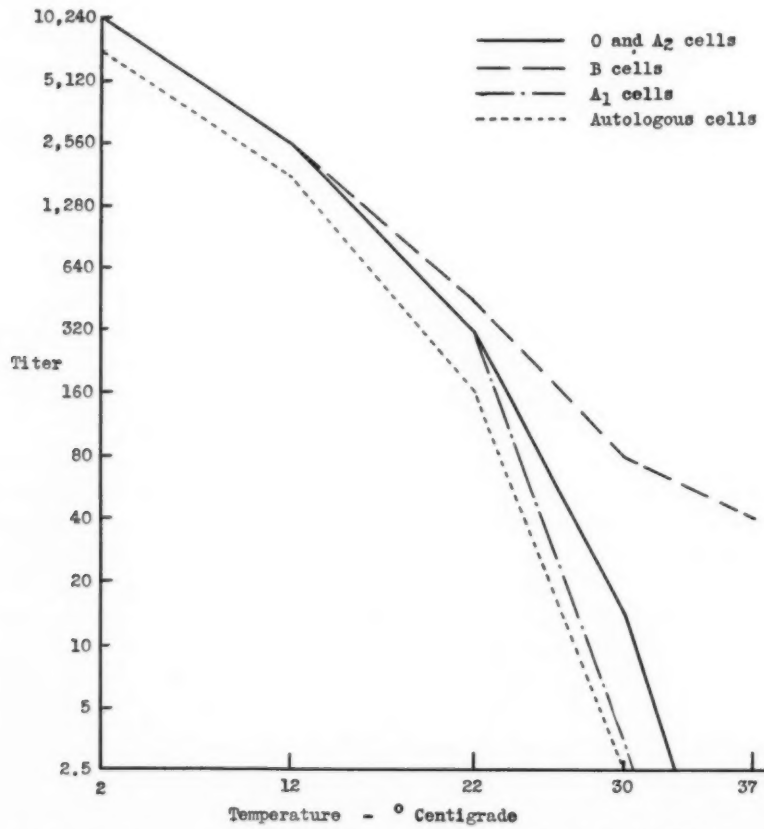

Fig. 1.—Average thermal curves for agglutination of O, A, B, and autologous cells by cold agglutinins of a human (G. C.) serum.

Blood samples were taken over a three months' period and the cold agglutinin titered at 2° with autologous, O, A, and B cells. All titers are expressed as the reciprocal of the highest final dilution of serum which gave agglutination. From 10,240 at the first bleeding on December 13, the titer dropped to 2560 after one week, 160 after three weeks, 20 after seven weeks, and 5 after three months. These and the following tests were done macroscopically using small serologic tubes and a final concentration of 0.15 per cent cells. Incubation times were those generally used at the various temperatures.[1]

Sera from the first and second bleedings were tested with autologous cells and with several O, A_1, A_2, and B cells at 37°, 30°, 22°, 12°, and 2°. Thermal curves for these cells are shown in Fig. 1. Titers for the autologous cells were

slightly lower than averages of the other A_1 cells. Titers for O and A_2 cells were the same, and at 30° the serum showed the properties of an α_2-agglutinin since the titer for O and A_2 cells was fourfold that of the A_1 titer. At 37° only the anti-B isoagglutinin was effective.

TABLE I

ADSORPTION AND ELUTION OF COLD AGGLUTININS

SERUM	AVERAGE 2° C. OLD AGGLUTININ TITERS FOR					
	AUTOLOGOUS CELLS (A_1)	O CELLS	A_1 CELLS	B CELLS	RABBIT CELLS	SHEEP CELLS
Unadsorbed	3840	5120	5120	5120	5120	160
Adsorbed with						
Autologous cells	<10	320	40	240	2560	60
O cells	160	40	80	80	2560	80
B cells	160	160	80	40	2560	20
Rabbit cells	320	1280	640	1280	20	20
Sheep cells	1280	1280	1280	1280	2560	<10
Eluate from						
Autologous cells	1280	3840	2560	2560	2560	80
O cells	1280	3840	1280	2560	2560	80
B cells	1280	2560	2560	2560	2560	40
Rabbit cells	1280	1280	640	640	5120	20
Sheep cells	1280	1280	640	1280	2560	80

Although the autoagglutinin acts on cells of all groups and is usually considered nonspecific, specific qualities in this case were shown by adsorption. The patient's serum in a 1:5 dilution was adsorbed at 2° for three successive times with autologous A, O, B, rabbit and sheep erythrocytes, and then tested at 2° with the various cells as shown in Table I. In some instances agglutinins were not completely removed, but in all cases titers after adsorption were lowest for the homologous cells used in the adsorption. Autoagglutinins for the patient's cells (A_1) were completely removed after three adsorptions, yet the serum contained cold agglutinins for human O and B, sheep, and rabbit cells and also, to a slight extent, for other A_1 cells. Similar reactions occurred following adsorptions with O or B cells, except that the titer differences in the adsorbed sera were less marked. Landsteiner[4] at an early date showed that cold agglutinins which had been adsorbed at low temperatures could be released from the cell by warming to body temperature or higher. By this method, purified solutions of the cold agglutinins were prepared from the various cells. Cells from the first 2° adsorption of the patient's serum were washed once at 2° with saline to remove traces of serum and then eluted at 50° in saline and centrifuged at that temperature. Eluates prepared in this way from autologous A, O, B, rabbit, and sheep cells were tested at 2° with the various cells with the results given in Table I. All elution fluids agglutinated each kind of cell with which they were tested, and titers were much the same for a particular type of cell regardless of the source of the eluate. The eluates exhibited no group specificity and even agglutinated erythrocytes from rabbits and sheep, species not closely related to human beings, although these species do have common heterogenetic antigens.

COMMENT

This patient seems of interest because of the unusually high titer of the cold agglutinin (10,240). When the blood was allowed to clot at room temperature

and the serum taken off at room temperature, the titer was somewhat lower (2560), probably due to adsorption of some of the agglutinin. Maximum titer could be obtained only when the serum was removed at 37° or higher. The cells of the patient reacted as normal A_1 cells, except that they were slightly less sensitive to agglutination than most other A_1 erythrocytes. The adsorption at 2° also showed a difference between the patient's cells and other A_1 cells since after adsorption with autologous cells the patient's serum still reacted with other A_1 cells.

Other members of the patient's family were examined since there is some evidence for familial occurrence of the cold agglutinin.[2] The father, mother, brother, sister, and daughter were examined and none of the sera in 1:5 dilution possessed autoagglutinins. This would probably be expected since the potent agglutinins of the patient were only temporary and diminished rapidly after the time of the first test.

Although autoagglutinins do not seem to be functional at body temperature, they are important as interfering agglutinins in blood grouping tests as usually done. The importance of the cross match of recipient's serum with donor's cells, and donor's serum with recipient's cells should be emphasized. When unusual reactions occur, such as agglutination of O cells at room temperature, the reaction is probably due to a cold- or autoagglutinin, and a correct typing and match will be given when the test is made at 37°. One other possibility should be kept in mind, however, and that is the case Morzycki[5] has described in which the blood (A_1) was deficient in the human species antigen and the serum contained agglutinins for O and A_2 cells which were functional at 37°. Presumably a group O donor would not be compatible for a blood of this sort.

REFERENCES

1. Kettel, K.: Studien über die Frage die Kalteagglutinination des Blutes bei Menschen, Acta path. et microbiol. Scandinav. **5**: 306, 1928.
2. Boxwell, W., and Bigger, J. W.: Autohemagglutination, J. Path. & Bact. **34**: 407, 1931.
3. Manheims, P. J., and Brunner, E. K.: Faulty Blood Grouping Due to Auto-agglutinins: An Unusual Case, J. A. M. A. **101**: 207, 1933.
4. Landsteiner, K.: Ueber Beziehungen zwischen dem Blutserum und den Korperzellen, München. med. Wchnschr. **50**: 1812, 1903.
5. Morzycki, J.: Ueber das Wesen der Agglutinine gegen das Menschenblut der O-Gruppe, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**: 80, 1934.

GLUCONIC ACID AS A URINARY ACIDIFYING AGENT IN MAN*

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THE fate of gluconic acid in the animal organism is a matter which is not fully established. In 1899 Salkowski[1] administered about 7 gm. of sodium gluconate daily to a rabbit for three consecutive days. Pentoses did not appear in the urine, and inasmuch as the urine during this time was strongly alkaline and contained little oxalic acid, he concluded that the gluconic acid was completely oxidized. In 1902 Mayer[2] published experiments in which he, too, was unable to recover any gluconic acid from the urine of rabbits after the oral administration of a very large dose, 7.1 gm. of sodium gluconate per kilogram of body weight. In 1905 Baumgarten[3] reported that 10 gm. doses of sodium gluconate in small normal, and depancreatized dogs, and 10 to 20 gm. doses in diabetic persons were also completely oxidized. In these experiments the urine failed to develop optical rotation or reducing power, and failed to yield combinations with phenylhydrazine. Several years later (1930), however, Hermann[4] reported a contrary result. He described an experiment in which he recovered 7.5 per cent of an oral dose of about 5.4 gm. of gluconic acid per kilogram of body weight in the urine of a rabbit. He suggested that this small recovery might be due to destruction of gluconic acid by microorganisms in the bowel prior to absorption. This, however, could not explain the results of experiments by other authors in which only small amounts of gluconic acid were recovered in the urine after the drug was administered subcutaneously.

After subcutaneous injections of sodium gluconate in doses of the order of 7 gm. per kg. in the rabbit, Mayer[2] recovered about 10 per cent in the urine; he stated that it existed in the urine in the form of saccharic acid. In 1911 Schott[5] confirmed the results of Mayer in part in that he also found that after subcutaneous large doses of sodium gluconate, total amounts of about 15 gm., a substance appeared in the urine which he believed, however, was not saccharic acid, but gluconic acid, and the amounts recovered represented from about 15 to 50 per cent of the dose injected.

Direct evidence concerning the fate of that part of gluconic acid which is not recovered in the urine does not exist. Schwarz[6] in 1903 showed that the oral administration of sodium gluconate sometimes reduced the excretion of acetone bodies in the urine and breath more effectively than even larger doses of glucose

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in diabetic patients. Baer and Blum[7] in 1911 failed to find diminished excretion of acetone bodies in phlorhizinized dogs and referred to this as an indication that gluconic acid is not converted to saccharic acid which they found does reduce the excretion of acetone bodies. Paderi[8] in 1918 concluded that gluconic acid is transformed into glucose because in his experiments the incubation of the acid with liver increased the glucose content, and the administration of gluconic acid to fasting rabbits increased the glycogen content of the liver.

The preponderance of evidence, therefore, seemed to indicate that while varying amounts of gluconic acid may appear in the urine after subcutaneous injection, little or none of even a large dose of it appears in the urine after its oral administration. It should be noted that the experiment in which some gluconic acid was found in the urine after the drug was administered orally involved a dose equivalent to about 300 gm. for an average adult of about 60 kg. Since only so little spilled over in the urine after such a massive amount, it cannot be stated whether any of it would be found in the urine after total doses of about 25 gm. a day which are given to human beings. In contrast to the above evidence on the conversion of gluconic acid into sugar and the failure of most investigators to isolate it in the urine after exceedingly large oral doses, Hermann[9] expressed the opinion that gluconic acid is an exception among the common organic acids in that it is not oxidized in the organism, although his experiments[4] presented no direct proof of this view.

Hermann[9] stated that acid soluble phosphate calculi placed in the bladder of the rabbit, were gradually reduced in size until they passed through the urethra, as the result of the daily feeding of gluconic acid for several weeks. How such calculi fared without gluconic acid was not stated. In these experiments massive doses of gluconic acid were given, 20 c.c. of a 5 per cent solution two to three times daily to a rabbit, equivalent to from 60 to 90 gm. or 1,200 to 1,800 c.c. of a 5 per cent solution of gluconic acid daily for the average man. He also stated that alkaline urine becomes acid after oral doses of gluconic acid but presented no controlled observations concerning this phenomenon. It should be noted that this observation is in conflict with that of Salkowski[1] who found that a dose of 7 gm. of sodium gluconate daily for three days left the urine of the rabbit strongly alkaline.

The papers of Hermann appear to have served as a basis for a recent report on gluconic acid and the delta lactone of gluconic acid as a urinary acidifying agent by Sisk and Toenhart.[10] They reported the effect on the pH of the urine in 7 human subjects, some with an infected and others with an uninfected urinary tract. They concluded that gluconic acid is a satisfactory urinary acidifying agent in man, and that its effects disappear rapidly so that frequent doses are necessary to maintain the low pH, namely, 4 to 6 gm. at intervals of two to four hours. They administered single doses of from 3 to 9 gm. every two hours, amounting to from 12 to 45 gm. daily, and stated that the minimum pH was usually reached in from one to three hours. This signifies, from the manner in which the doses were given, a minimum pH after total doses of from 3 to 18 gm. of gluconic acid.

In 4 of the 7 cases studied by Sisk and Toenhart there were no controls, the first pH being determined one hour after the first dose of the drug. In 2 cases the control consisted of one pH value before each day's treatment. When their results are examined, using in each case the first pH of the day as a control,* one finds that of 35 such days the urine was most alkaline in the first specimen on twenty days, and most alkaline in at least one specimen during the course of the treatment on fifteen days. It is well known that the pH shows wide fluctuations throughout the day, and the control course under the conditions of their experiments was reported in only one patient, and in that case (Case 2), the pH averaged 6.4 (range 5.7 to 6.7) in the first two-day control period, and 6.1 (range 4.9 to 6.9) in the first two-day treatment period. Such a difference is also seen when the two control days are compared with each other, namely, 6.2 for the first day and 6.5 for the second day, and in this patient in a series of eight treatment days with the same daily doses, the average of the daily pH values varied irregularly from 5.1 to 6.1. The pH of the urine taken at 9 A.M. (one hour after the first dose) on eight different days varied in this individual from 5.2 to 6.9. In their Case 5, for example, the first specimens of urine of the two-day period of gluconic acid treatment averaged 6.7, while the first specimens of the four-day period of ammonium nitrate treatment averaged 5.6. These illustrations suffice to indicate the danger of isolated pH values as controls. Their results, therefore, do not supply sufficient proof that gluconic acid is a useful urinary acidifying agent. The present experiments were undertaken to re-examine this question.

TABLE I

COMPARISON OF pH VALUES OF URINE WITH GLASS AND QUINHYDRONE ELECTRODES

SUBJECT	GLASS	QUINHYDRONE*	DIFFERENCE
T	4.88	5.03	0.15
H	5.15	5.34	0.19
M	5.00	5.16	0.16
J	5.44	5.54	0.10
T	4.82	4.93	0.11
M	5.04	5.17	0.13
J	5.42	5.50	0.08
H	5.03	5.06	0.03
M	4.86	5.02	0.16
J	5.27	5.29	0.02
C	5.21	5.36	0.15

*The readings were made promptly after the quinhydrone was added.

EXPERIMENTAL

More than 350 specimens of urine were examined for specific gravity and pH. The pH was determined by the glass electrode in one series, and by the quinhydrone electrode in the other series. Eleven specimens of urine examined by both methods showed uniformly slightly higher values with the quinhydrone electrode (Table I). The latter is not quite as satisfactory as the glass electrode for urine because of the tendency to drifting if there is delay in making the reading.

*The first pH in the records was either before treatment or after one dose of gluconic acid.

In one group of experiments 5 normal subjects were used, and a determination was made of the effect of a large dose of gluconic acid on the pH of the urine secreted during the first four-hour period after the dose. The plan included the following: constant breakfast at about 8 A.M. (such as coffee, or roll and coffee); the bladder emptied at 9 A.M. and voiding discarded; a glass of water at 9 A.M. (in controls), or 15 gm. of the gluconic acid* washed down with the glass of water (on the treatment days); no food, drink, or voiding until 1 P.M. In all, about 70 daily specimens of urine were examined during this part of the work. The urine was examined as soon after voiding as it reached room temperature (fifteen or twenty minutes).

In the second group of experiments an attempt was made in another way to determine the effect of gluconic acid on the acidity of the urine, namely, to compare the average as well as the range of several pH values on one or more control days with those on one or more treatment days. Observations were made on 16 persons,† 8 of whom were confined to bed in the hospital, and 8 of whom were ambulant. In 7 of the bed patients there were various urologic conditions with urinary infection. These patients were selected in part because of the high pH values of their urine before treatment, which served as a more satisfactory control for the effects of an acidifying agent. The diets of the ambulant patients, as well as their urinary volumes, varied considerably, but in the case of the bed patients they were the regular ward diets of the hospital, which are more constant. The daily urinary output in these patients varied between approximately 1,500 and 3,000 c.c., except in one patient (Case 2) in whom the daily urine volume was only about 900 c.c. The delta lactone was administered in 5 gm. doses at two-hour intervals up to total doses of from 15 to 25 gm. daily, and subsequently in 10 gm. doses every two hours up to total doses of from 20 to 50 gm. daily. In one case a single dose of 15 gm. was given. In another case 2 doses of 25 gm. each were given at two-hour intervals. In a few instances the delta lactone was dissolved in water and diluted to an approximately 2 to 3 per cent solution for administration. This proved rather irritating to the mouth, however, and in the majority of instances it was found more satisfactory to take it as the dry powder followed by a glass of water. The pH was determined with the quinhydrone electrode in this series. The specimens of urine were collected at two-hour intervals from 7 A.M. to 5 P.M., both on control days and treatment days, the specimens being kept on ice, and the pH values determined in less than an hour after collection. The specific gravity of every specimen was also determined. Since most of the subjects received abundant fluids, the specific gravity of the urines was fairly low (1.002 to 1.021) and appeared to vary without relation to the pH.

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*This was given as the delta lactone. It was supplied as the preparation of glucono-delta-lactone by Chas. Pfizer & Co., Inc.

†We are deeply indebted to Dr. Charles G. Child of the Department of Surgery for making available to us 7 urologic patients as subjects, and for his help in establishing the necessary routine in these cases; also to Dr. Ade T. Milhorat for his patient who was controlled on a strictly constant diet in connection with his work on progressive muscular dystrophy.

The following is a protocol of a typical experiment (Patient 2):

DATE	TIME OF SPECIMEN	7	9	11	1	3	5
			Control Days				
10/12/38	pH	–	7.0	7.0	6.7	7.0	6.9
	Specific gravity	–	1.008	1.006	1.004	1.004	1.000
10/13/38	pH	6.9	7.6	6.9	7.5	7.1	7.1
	Specific gravity	1.008	1.012	1.012	1.012	1.010	1.012
			Treatment Days				
10/14/38	pH	9.2	8.8	8.8	8.9	9.7	9.0
	Specific gravity	1.027	1.009	1.010	1.010	1.010	1.015
Dose of drug at		7	9	11	1	3	
10/15/38	pH	8.7	8.6	9.1	8.9	6.6	9.1
	Specific gravity	1.020	1.021	1.009	1.006	1.004	1.010
Dose of drug at		7	9	11	1	3	
			Control Days				
10/19/38	pH	9.4	8.9	8.9	8.7	8.8	9.0
	Specific gravity	1.006	1.008	1.004	1.002	1.006	1.008
10/20/38	pH	7.6	7.6	7.3	7.2	6.9	–
	Specific gravity	1.010	1.007	1.008	1.010	1.008	–
			Treatment Day				
10/21/38	pH	7.0	7.4	7.0	7.5	6.9	7.0
	Specific gravity	1.008	1.008	1.010	1.010	1.006	1.010
*Dose of drug at		7	9	11	1		

*Dose of drug was 5 gm. during first two days of treatment and 10 gm. during the third day. It was discontinued because of nausea and diarrhea.

RESULTS

The results obtained in the first series of experiments are shown in Table II. The indications are unequivocal in showing that the urine secreted during the first four-hour period after a very large dose of the delta lactone of gluconic acid is more acid than control specimens obtained under similar conditions. The pH of the gluconic acid periods never reached the high values of the controls, although they did not go far below the lowest values of the controls. That the urine resists acidification below the lowest normal pH values has been observed by others in connection with other urinary acidifying agents (Johnston, 1932[11]). Three of the 5 subjects developed abdominal cramps and diarrhea. In one patient vomiting occurred. The specific gravity of the urine showed no consistent relationship to the changes in the pH.

The essentials of the results in the second series of experiments are summarized in Table III. Since the values after the 10 gm. doses were similar to those after the smaller ones, they have been considered together in the table. In the control periods the subjects had been without drug for at least twenty-four hours, and in the values of the treatment period the last specimens included are those taken no later than two hours after the last dose of the drug.

In 8 of the 16 patients, the urine became more acid, and in the other half it became more alkaline during the period of treatment with gluconic acid. The failure of this series to reveal clearly the acidifying action of gluconic acid may

TABLE II

EFFECT OF DELTA LACTONE OF GLUCONIC ACID ON pH§ OF URINE SECRETED DURING FIRST FOUR HOURS AFTER A DOSE OF 15 GM.

SUBJECT T DATE	pH		SPECIFIC GRAVITY		SUBJECT M DATE	pH		SPECIFIC GRAVITY	
	CONTROLS	AFTER DRUG	CONTROLS	AFTER DRUG		CONTROLS	AFTER DRUG	CONTROLS	AFTER DRUG
12/3	6.0	–	1.007	–	12/2	7.0	–	1.010	–
12/20	5.9	–	1.008	–	12/3	6.2	–	1.017	–
12/19	5.8	–	1.020	–	12/19	5.7	–	1.017	–
12/21	5.6	–	1.012	–	12/21	5.6	–	1.028	–
12/2	5.6	–	1.014	–	12/29	5.4	–	1.024	–
12/1	5.2	–	1.011	–	12/30	5.4	–	1.026	–
12/6	4.9	–	1.010	–	12/20	5.4	–	1.016	–
1/5	–	4.9	–	1.016	12/1	5.2	–	1.026	–
1/9	–	4.9	–	1.007	12/22	–	5.2	–	1.020
12/9	–	4.9	–	1.014	12/9	5.1	–	1.016	–
12/7	–	4.8	–	1.016	12/28	–	5.1†	–	1.020
12/10	–	4.8	–	1.010	12/27	–	5.0*	–	1.028
					12/6	–	5.0	–	1.025
					12/7	–	5.0	–	1.025
					12/8	–	4.9	–	1.018

SUBJECT J DATE	pH		SPECIFIC GRAVITY		SUBJECT H DATE	pH		SPECIFIC GRAVITY	
	CONTROLS	AFTER DRUG	CONTROLS	AFTER DRUG		CONTROLS	AFTER DRUG	CONTROLS	AFTER DRUG
12/1	7.2	–	1.018	–	12/2	5.2	–	1.024	–
12/30	6.8	–	1.024	–	12/16	5.2	–	1.024	–
12/3	6.2	–	1.024	–	12/19	5.2	–	1.022	–
12/16	5.9	–	1.019	–	12/20	5.2	–	1.023	–
12/27	5.8	–	1.016	–	12/6	–	5.2*	–	1.026
12/2	5.8	–	1.015	–	12/1	5.1	–	1.027	–
12/9	5.5	–	1.018	–	12/28	5.1	–	1.018	–
12/19	5.5	–	1.020	–	12/29	–	5.1†	–	1.016
12/22	–	5.5*	–	1.022	12/9	–	5.1*	–	1.026
12/20	5.5	–	1.020	–	12/22	–	5.1*	–	1.028
12/21	5.5	–	1.018	–	12/3	5.1	–	1.022	–
12/28	–	5.5‡	–	1.020	12/17	5.1	–	1.017	–
12/29	5.4	–	1.020	–	12/21	5.1	–	1.019	–
12/6	–	5.4	–	1.016	12/8	–	5.0	–	1.024
12/23	–	5.4	–	1.018	12/23	–	5.0*	–	1.031
12/7	–	5.4	–	1.018					
12/8	–	5.3	–	1.020					

SUBJECT C DATE	pH		SPECIFIC GRAVITY	
	CONTROLS	AFTER DRUG	CONTROLS	AFTER DRUG
12/23	7.1	–	1.019	–
12/22	6.9	–	1.011	–
12/29	6.3	–	1.025	–
12/9	5.9	–	1.010	–
12/30	5.9	–	1.020	–
1/5	–	5.4	–	1.019
1/6	–	5.3	–	1.012
12/8	5.2	–	1.012	–
12/16	–	5.2	–	1.005
12/21	5.1	–	1.012	–

*Diarrhea caused by the drug.
†Dose only 10 gm.
‡Vomited within 1 hr. 15 min. after dose.
§The pH in this series was determined by the glass electrode.

in part be due to the smaller single doses and to the masking effect of diet during the day.

Eleven of the 16 patients developed diarrhea with or without nausea at some time in the course of the use of the gluconic acid; in 6 of these it occurred with the 5 gm. doses.

TABLE III

NO.	SUBJECT	SEX	AGE	DIAGNOSIS	INFECTED URINE	TOTAL DAILY DOSE OF LACTONE (GM.)			NO. OF CONTROL DAYS	NO. OF SPECIMENS IN CONTROLS	NO. OF SPECIMENS AFTER DRUG	pH DETERMINATIONS				CHANGE IN pH DURING TREATMENT	REMARKS
						FIRST DAY (5 GM. DOSES EACH)	SECOND DAY (5 GM. DOSES EACH)	THIRD DAY (10 GM. DOSES EACH)				RANGE IN CONTROLS	RANGE AFTER DRUG	AVERAGE IN CONTROLS	AVERAGE AFTER DRUG		
1	Mal	M	64	Renal calculus	+	25	25	50	4	24	15	8.1-6.4	9.7-6.2	6.9	7.1	+0.2	Diarrhea after 50 gm.
2	Mc	M	30	Pyonephrosis and nephritis	+	25	25	40	4	19	14	9.0-6.9	9.7-6.6	7.6	8.1	+0.5	Diarrhea and nausea after 25 gm.
3	Te	F	26	Hypernephroma	+	25	25	30	4	18	13	7.7-6.7	7.0-5.7	7.5	6.6	-0.9	Diarrhea after 30 gm.
4	Ko	F	43	Renal calculi	+	25	25	50	4	18	15	7.8-6.5	7.4-6.7	7.2	7.1	-0.1	
5	Ba	M	70	Papillomatosis of bladder; hydronephrosis	+	-	-	30	2	9	3	7.6-6.0	7.1-6.5	6.9	6.8	-0.1	Diarrhea after 20 gm.
6	Za	M	30	Tuberculosis of bladder; prostate, and kidney	+	25	25	-	2	10	10	8.6-6.4	8.2-6.9	7.7	7.8	+0.1	Diarrhea after 25 gm.
7	Ha	M	73	Benign hypertrophy of prostate; transurethral resection	+	-	-	50	2	10	5	7.3-6.8	6.9-6.4	7.0	7.1	+0.1	Diarrhea and nausea after 40 gm.
8	Gu	M	24	Normal	0	20	-	-	1	4	4	7.6-5.6	7.2-6.5	6.7	6.9	+0.2	Diarrhea after 20 gm.
9	Ci	F	27	Normal	0	25	-	-	2	5	5	7.2-5.2	6.8-6.3	5.8	6.5	+0.7	
10	He	M	41	Normal	0	15	20	20	1	4	11	5.8-5.3	6.2-5.4	5.6	5.7	+0.1	Diarrhea after 15 gm.
11	Me	M	10	Progressive muscular dystrophy	0	15	25	50*	2	7	9	7.2-6.1	6.7-5.5	6.6	6.0	-0.6	Diarrhea after 25 gm.
12	Tr	F	37	Normal	0	20	20	-	3	10	10	7.3-5.2	6.2-5.0	6.2	5.2	-1.0	
13	Mo	M	30	Normal	0	-	-	15 (1 dose)	4	4	2	5.6-5.2	5.8-5.3	5.4	5.6	+0.2	
14	Go	M	41	Normal	0	15	-	-	1	2	3	-	5.4-5.0	5.3	5.2	-0.1	
15	Mat	M	42	Normal	0	15	-	-	1	4	3	6.5-5.3	5.4-5.0	5.8	5.2	-0.6	Diarrhea after 15 gm.
16	Jo	M	39	Normal	0	25	-	-	1	4	5	7.2-6.5	6.5-5.8	6.9	6.1	-0.8	Diarrhea after 15 gm.

*Two doses of 25 gm. each.

SUMMARY AND CONCLUSIONS

Under controlled experimental conditions gluconic acid given in the form of the delta lactone in large doses reduces to low levels the pH of the urine in man. Its acidifying action under the ordinary conditions of diet and fluid intake, however, appears limited, since under these conditions smaller doses yielded equivocal results on the pH, and even these caused a high incidence of gastrointestinal disturbance. From the practical standpoint, further experimentation is necessary to establish the conditions under which the delta lactone of gluconic acid may prove useful as a urinary acidifying agent.

REFERENCES

1. Salkowski, E.: Ueber das Vorkommen von Pentosen im Harn, Ztschr. f. physiol. Chem. **27**: 507, 1899.
2. Mayer, P.: Experimentelle Untersuchungen über Kohlenhydratsäuren, Ztschr. f. klin. Med. **47**: 68, 1902.
3. Baumgarten, O.: Ein Beitrag zur Kenntnis des Diabetes mellitus, Ztschr. f. exper. Path. u. Therap. **2**: 53, 1905.
4. Hermann, S.: Zur Pharmakologie der Gluconsäure, Arch. f. exper. Path. u. Pharmakol. **154**: 175, 1930; Ibid. **154**: 143, 1930.
5. Schott, E.: Über Verhalten von Glyconsäure und Zuckersaure im Organismus, Arch. f. exper. Path. u. Pharmakol. **65**: 35, 1911.
6. Schwarz, L.: Untersuchungen über Diabetes, Deutsches Arch. f. klin. Med. **76**: 233, 1903.
7. Baer, J., and Blum, L.: Über die Einwirkung chemischer Substanzen auf die Zuckerausscheidung und die Acidose, Arch. f. exper. Path. u. Pharmakol. **65**: 1, 1911.
8. Paderi, C.: Sur la mode de se comporter de l'acide glyconique dans l'organisme (Abst.), Zentralbl. f. Biochem. u. Biophys. **19**: 622, 1918.
9. Hermann, S.: Methode zur Auflösung von Phosphatkonkremanten der Harnwege, München. med. Wchnschr. **82**: 540, 1935.
10. Sisk, I., and Toenhart, O.: Gluconic Acid as a Urinary Acidifying Agent, J. Urol. **39**: 699, 1938.
11. Johnston, J. M.: A Review of the Pharmacology of Urinary Antiseptics, Lancet **1**: 54, 1932.

STAPHYLOCOCCUS EPIDERMIS ALBUS*

Cultural and Immunologic Reactions of Large and Small Colony Types

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IT HAS long been noted that upon the swabbing of skin surfaces and subsequent plating on agar, both large and small staphylococcus colonies appear. After a thorough washing of the skin with soap and water, the number of small colony staphylococci increases. Colonies measuring 1 mm. or less in diameter were classified as "small colony type," while those measuring from 2 mm. to 4 mm. were classified as "large colony type." Growth produced by the small colony type was always rather scanty and frequently failed to survive transfer from the original plates.

Technique.—The skin was thoroughly washed with soap and water and dried with sterile gauze. A sterile moist swab was used to swab the skin area.

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It was then thoroughly rubbed over an agar plate. The plate was incubated for forty-eight hours. Only albus colored colonies of both sizes were selected for study. Subcultures were made on agar plates to insure purity. After a forty-eight-hour incubation period, 25 typical colonies, measuring 2 mm. to 4 mm., and 25 colonies, measuring 1 mm. or less, were picked and transferred to agar slants.

TABLE I

STAPHYLOCOCCUS EPIDERMIS ALBUS (LARGE COLONY TYPES)
Isolated From the Surface of the Skin

STRAIN	LITMUS MILK	NITRATE	GELATIN	DEXTROSE	MANNITE	MALTOSE	LACTOSE	RAFFINOSE	SUCROSE	INULIN	GRAM STAIN
1	+	+	+	+	−	+	+	−	+	−	+
2	−	−	−	+	−	+	−	−	+	−	+
3	−	+	−	+	1+	+	−	−	+	−	+
4	−	+	−	+	−	+	−	−	+	−	+
5	−	+	+	+	−	+	−	−	+	−	+
6	+	−	+	+	−	+	−	−	+	−	+
7	−	+	−	+	−	+	+	−	+	−	+
8	−	+	+	+	−	−	−	−	+	−	+
9	+	+	+	+	−	+	+	−	+	−	+
10	−	−	−	+	−	+	+	−	+	−	+

TABLE II

STAPHYLOCOCCUS EPIDERMIS ALBUS (SMALL COLONY TYPES)
Isolated From the Surface of the Skin

STRAIN	LITMUS MILK	NITRATE	GELATIN	DEXTROSE	MANNITE	MALTOSE	LACTOSE	RAFFINOSE	SUCROSE	INULIN	GRAM STAIN
1	−	+	−	+	1+	1+	−	−	+	−	+
2	−	+	+	+	−	1+	−	−	+	−	+
3	+	−	+	+	−	+	+	−	+	−	+
4	+	+	+	+	−	+	+	−	+	−	+
5	−	+	−	+	1+	+	−	−	+	−	+
6	−	+	−	+	−	1+	−	−	1+	−	+
7	−	+	−	+	−	1+	−	−	1+	−	+
8	−	+	−	+	−	+	−	−	1+	−	+
9	−	+	+	+	−	+	−	1+	−	−	+
10	−	+	+	+	−	1+	−	−	1+	−	+

TABLE III

STAPHYLOCOCCUS EPIDERMIS ALBUS (LARGE COLONY TYPES)
Biochemical Groups

GROUP	NUMBER OF STRAINS	LITMUS MILK	NITRATE	GELATIN	DEXTROSE	MANNITE	MALTOSE	LACTOSE	RAFFINOSE	SUCROSE	INULIN
1	8 (44)*	+	+	+	+	−	+	+	−	+	−
2	7 (50)*	−	−	−	+	+	+	+	−	+	−
3	3 (41)*	−	+	+	+	−	−	−	−	+	−
4	7	−	+	−	+	−	+	+	−	+	−

*Strains used for immunologic experiments.

TABLE IV

STAPHYLOCOCCUS EPIDERMIS ALBUS (SMALL COLONY TYPES)

Biochemical Groups

GROUP	NUMBER OF STRAINS	LITMUS MILK	NITRATE	GELATIN	DEXTROSE	MANNITE	MALTOSE	LACTOSE	RAFFINOSE	SUCROSE	INULIN
1	5	+	+	+	+	−	+	+	−	+	−
2	6 (11s)*	±	−	+	+	−	+	+	−	+	−
3	6 (22s)*	−	+	+	+	−	±	−	−	+	−
4	8 (18s)*	−	+	−	+	±	±	−	−	±	−

*Strains used for immunologic experiments.

TABLE V

	LACTOSE	MALTOSE	DEXTROSE	LITMUS MILK	PER CENT
Large staphylococci colonies (skin)	+	+	+	+	28
Large staphylococci colonies (skin)	−	+	+	−	20
Large staphylococci colonies (skin)	−	−	+	−	8
Large staphylococci colonies (skin)	+	+	+	−	36
Large staphylococci colonies (skin)	−	+	+	+	8

TABLE VI

	LACTOSE	MALTOSE	DEXTROSE	LITMUS MILK	PER CENT
Small staphylococci colonies (skin)	+	+	+	+	22
Small staphylococci colonies (skin)	−	+	+	−	50
Small staphylococci colonies (skin)	−	−	+	−	18
Small staphylococci colonies (skin)	+	+	+	−	9

TABLE VII

STAPHYLOCOCCUS EPIDERMIS ALBUS (SKIN)	REDUCTION OF NITRATES		LIQUEFACTION OF GELATIN		ACID IN MANNITE	
	Per Cent		*Per Cent*		*Per Cent*	
	Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
Large colony type	64	36	52	48	28	72
Small colony type	68	32	64	36	22	78

TABLE VIII

AGGLUTINATION REACTIONS

Large and Small Staphylococci Colonies

	SERUM 44	SERUM 50	SERUM 18S	SERUM 11S	SERUM 22S
Strain 44	640	2560	320	320	160
Strain 50	40	640	0	40	20
Strain 41*	2560	40	320	80	320
Strain 18s	320	2560	640	640	320
Strain 11s	320	20	0	40	0
Strain 22s	20	80	80	−†	80

Numbers 11s, 18s, and 22s are small colony types.
Numbers 44, 50, and 41 are large colony types.
*Rabbit died five days after last injection; hence no serum was obtained.
†Rabbit died five days after trial bleeding. Not enough serum remained to run test.

In this work we were interested in determining whether there are any marked differences in cultural and immunologic reactions between the large and small colony staphylococci found on the skin.

Biochemical Reactions.—Subcultures were made in litmus milk, nitrate peptone solution, gelatin, dextrose, mannite, maltose, lactose, raffinose, sucrose, and inulin. Table I shows the cultural reactions of the first ten strains in the large colony group, and Table II shows those of the first ten in the small colony group. The cultures were grouped according to biochemical reactions, and Tables III and IV show the number belonging to each group. Throughout the work it was evident, as is shown in the tables, that the small colony types are not as active biochemically as the large colony types. Tables V and VI show that 64 per cent of the large type ferment lactose as compared with only 31.8 per cent of the small type. Table VII shows the small type to be more active gelatin liquefiers than the large type.

Immunologic Reactions.—Three strains were selected from the large colony types (No. 44 from group 1, No. 50 from group 2, and No. 41 from group 3), and three strains from the small colony types (No. 11s from group 2, No. 22s from group 3, and No. 18s from group 4). Rabbits were selected for the immunologic work. Heavy saline suspensions of the living organisms were injected intravenously, three injections being given in all. Ten days after the last injection, trial bleedings were made and the titers of sera tested. The rabbits were then bled to completion. During the course of the work one death occurred (No. 41) five days after the last injection. The cause could not be determined. Another rabbit (No. 11s) died following the trial bleeding. No serum was obtained from rabbit No. 41, but enough was obtained from No. 11s in the trial bleeding to suffice for all but one test. The rabbits showed more reaction after injection with the large type colony staphylococci than the small type colony staphylococci.

The agglutination reactions are summarized in Table VIII. It is evident from these results that all of these strains are related to each other. The large colony type have broader antigenic relationships; hence they may be considered the parent strain. Strain 50 is type specific, while strains 44, 41, and 18s have wide antigenic relationships. The agglutinins in antisera 44 and 50 have a wide band of activity.

SUMMARY

The results of this work indicate:

(1) All of these strains are related to each other.

(2) The large colony types are more highly antigenic, have wider antigenic relationships and therefore may be considered the parent strain.

(3) The large colony types are more active biochemically than the small types.

(4) The growth produced by the small colony type is always limited as compared to that of the large colony type.

REFERENCES

Arnold, L., Gustafson, C. J., Hull, T. G., Montgomery, B. E., and Singer, Charlotte: The Self-Disinfecting Power of the Skin as a Defense Against Microbic Invasion, Am. J. Hyg. **11**: 345, 1930.

Colebrook, L., and Maxted, W. R.: Antisepsis in Midwifery, J. Obst. & Gynaec. Brit. Emp. **40**: 966, 1933.
Cowan, S. T.: The Classification of Staphylococci by Precipitation and Biological Reactions, J. Path. & Bact. **46**: 31, 1938.
Hine, T. G. M.: Serological Classification of the Staphylococci, Lancet **2**: 1380, 1922.
Julianelle, L. A.: Studies of Hemolytic Staphylococci Agglutinations, J. Infect. Dis. **31**: 256, 1922.
Julianelle, L. A., and Wieghard, C. W.: Immunological Specificity of Carbohydrates Derived From Staphylococci, Proc. Soc. Exper. Biol. & Med. **31**: 947, 1933.
Julianelle, L. A., and Wieghard, C. W.: The Immunological Specificity of Staphylococci. II. The Chemical Nature of the Soluble Specific Substances, J. Exper. Med. **62**: 31, 1935.
Klopstock and Bochenheimer: Beitrag zur Agglutination der Staphylokokken, Arch. f. klin. Chir. **72**: 325, 1904.
Koch, Joseph: Ueber das Vorkommen Pathogener Staphylokokken auf der Korperoberflaeche des Menschen und Seiner Umgebung, Ztschr. f. Hyg. u. Infektionskr. **58**: 287, 1908.
Kutscher and Konrich: Untersuchungen ueber die Beziehungen von Haemolysinbildung und Agglutinobilitat der Staphylokokken, Ztschr. f. Hyg. u. Infekionskr. **48**: 249, 1904.
Montgomery, B., and Gustafson, C. J.: Classification of Staphylococcus Epidermis Albus From Human Skin, Proc. Soc. Exper. Biol. & Med. **27**: 365, 1930.
Winslow, C. E. A., Rothberg, Wm., and Parsons, Eliz. I.: Notes on the Classification of the White and Orange Staphylococci, J. Bact. **5**: 145, 1920.
Bergey, D. H.: Manual of Determinative Bacteriology, Baltimore, 1939, Williams & Wilkins Co.

A STUDY OF ENZYMES IN NORMAL AND PATHOLOGIC CEREBROSPINAL FLUID*

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ACKNOWLEDGE of the enzymes of the cerebrospinal fluid may lead to a clearer understanding of the physiology of the central nervous system and may also be of clinical value in diseases of the central nervous system. It is not surprising, therefore, that the study of the enzymes of the cerebrospinal fluid has attracted a number of workers.[1, 2] As yet, however, no adequate correlation has been made between the occurrence of enzymes in the cerebrospinal fluid and the condition of the central nervous system.[2] Some workers have limited themselves to the study of only one or two enzymes[3-5] or to only a few pathologic conditions, such as mental diseases.[6] Others have studied the factors which regulate the permeability of the meninges[7-9] and have not been interested in the enzymes of the cerebrospinal fluid.

In an attempt to investigate the relationship between the condition of the central nervous system and the distribution of enzymes in the cerebrospinal fluid, we studied the enzymatic activity of over one hundred specimens of normal and pathologic cerebrospinal fluid. The enzymes studied were trypsin,† phosphatase, lipase, tributyrinase, esterase, and amylase. Anti-

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†Throughout the paper certain conventions will be used with respect to the nomenclature of the enzymes. By trypsin is meant the enzyme system which catalyzes the breakdown of denatured hemoglobin in a slightly alkaline medium. The identity of the enzyme with pancreatic trypsin or other tryptic enzymes is not discussed here. The term "trypsin" has been chosen for convenience. Similarly, by lipase is meant the enzyme system which hydrolyzes olive oil, by tributyrinase, the enzyme which hydrolyzes tributyrin; by esterase, the enzyme which hydrolyzes ethyl butyrate, etc.

trypsin, although not an enzyme, was studied in connection with trypsin. Cell counts were done in each instance and determinations of the sugar, chlorides, total protein and nonprotein nitrogen contents of the fluids were made whenever possible.* When spinal punctures were necessary for diagnostic or therapeutic purposes, specimens of cerebrospinal fluid were obtained from hospital patients, most of whom were children. Most of the punctures were lumbar; when ventricular or cisternal punctures were resorted to, they are so marked in the tables. The fluids were withdrawn after an overnight fast, and enzymatic and chemical studies were begun within one or two hours. Unless otherwise indicated, the fluids were clear and colorless.

METHODS

Trypsin was determined by the method of Anson and Mirsky[10] with modifications made necessary by the nature of the material analyzed. In this method the amount of tyrosine formed as a result of the digestion of a hemoglobin substrate by a tryptic enzyme is measured colorimetrically. The method as modified for the purpose of this work is here presented.

One cubic centimeter of cerebrospinal fluid was added to 5 c.c. of hemoglobin substrate and the mixture was incubated at 37° C. for twenty-four hours. An incubation period of four hours is sufficient to yield results, but the longer incubation period gives greater precision because of the larger amounts of tyrosine formed. Ten cubic centimeters of 5 per cent trichloracetic acid were then added to the digestion mixture to precipitate the undigested hemoglobin and to stop the enzyme reaction. After five minutes, the solution was filtered. To 5 c.c. of filtrate were added 10 c.c. of 0.50 N sodium hydroxide and 3 c.c. of phenol reagent diluted 1:3. The color which developed was compared in the colorimeter with a suitable tyrosine standard set at 20. The standards were prepared by adding to 5 c.c. of tyrosine solution containing 0.10, 0.15, or 0.25 mg. of tyrosine, 10 c.c. of 0.5 N sodium hydroxide and 3 c.c. of diluted phenol reagent. A blank, or zero time determination, was run simultaneously by adding 1 c.c. of cerebrospinal fluid to 5 c.c. of substrate solution at 37° C., and adding immediately 10 c.c. of 5 per cent trichloracetic acid. The difference between the number of millimoles of tyrosine found after incubation and without incubation was taken as the measure of the tryptic activity of the cerebrospinal fluid. Calculations of the tryptic activity were made, using modifications of the methods of calculation of Anson and Mirsky similar to those of Hecht and Civin.[11] These modifications were necessary because of the wide variation of the amounts of trypsin found in cerebrospinal fluid, requiring the use of more than one standard. The number of millimoles of tyrosine found colorimetrically can be written:[11, 12]

$$(1)\ \text{mM. Tyrosine} = \frac{16}{5} \times \frac{1}{181} \times \frac{20}{x}\ (\text{mg. tyrosine standard}) - \text{reagent. blank}$$

The number of trypsin units per 100 c.c. of cerebrospinal fluid is therefore,

$$(2)\ \text{mM. Tyrosine after 24 hours} - \text{mM. tyrosine at 0 hours} = \text{T.U. per 100 c.c.} = \frac{1600}{905}\left[\frac{20}{x_{24}}\ (\text{mg. } T^{24}_{St.}) - \frac{20}{x_0}\ (\text{mg. } T^{0}_{St.})\right],$$

*Studies were also made of the calcium and phosphorus contents. These have been reported elsewhere (J. LAB. & CLIN. MED. 24: 609, 1939).

where mg. $T\frac{24}{St.}$ = the number of milligrams of tyrosine in the standard required for the twenty-four-hour reading, and mg. $T\frac{0}{St.}$ is the number of milligrams of tyrosine in the standard required for the initial reading. x_{24} and x_0 are the respective colorimetric readings. For example, if the 0.25 mg. tyrosine standard is required at twenty-four hours, and 0.10 mg. standard for the initial reading, the number of trypsin units per 100 c.c. is:

$$(3)\text{T.U.} = 1.77\left[\frac{20}{x_{24}}(0.25) - \frac{20}{x_0}(0.10)\right]$$

$$= 1.77\left[\frac{5.0}{x_{24}} - \frac{2.0}{x_0}\right]$$

Antitrypsin was determined by measuring the effect of the presence of cerebrospinal fluid on the activity of a commercial trypsin preparation. To 5 c.c. of hemoglobin substrate were added 1 c.c. of a solution (1:1000) of commercial trypsin and 1 c.c. of water or of cerebrospinal fluid. The percentage inhibition of the particular trypsin used was taken as the measure of the antitryptic power of the cerebrospinal fluid. The decrease in millimoles of tyrosine formed in the presence of cerebrospinal fluid is given by the equation:

$$(4)\ \text{mM. Tyrosine} = \frac{1700}{905}\left[\frac{20}{x_{H_2O}}(\text{mg. } T^{H_2O}_{St.}) - \frac{20}{x_{CSF}}(\text{mg. } T^{CSF}_{St.})\right],$$

where mg. $T\frac{H_2O}{St.}$ and mg. $T\frac{CSF}{St.}$ are the numbers of milligrams of tyrosine in the standards required when water and cerebrospinal fluid, respectively, were added to the hydrolysis mixture; xH_2O, and x_{CSF} are the corresponding colorimetric readings. The number 1700 appears in the formula instead of 1600 because the digestion mixture on addition of 5 per cent trichloracetic acid contains 17 c.c. instead of 16 c.c. as in the determination of trypsin. The percentage decrease in millimoles of tyrosine formed is then:

$$(5)\ \text{Antitrypsin units} = \text{per cent inhibition} =$$

$$\frac{\frac{20}{x_{H_2O}}(\text{mg. } T^{H_2O}_{St.}) - \frac{20}{x_{CSF}}(\text{mg. } T^{CSF}_{St.})}{\frac{20}{x_{H_2O}}(\text{mg. } T^{H_2O}_{St.}) - \text{reagent blank}} \times 100 .$$

The phosphatase activity of the cerebrospinal fluid was determined by the method of Bodansky,[12] which depends on the determination of the inorganic phosphorus liberated from a phosphoric acid ester by the action of the enzymatic material. Because of the small phosphatase activity usually found in the cerebrospinal fluid, a few slight modifications of the above-mentioned method were necessary. Ten cubic centimeters of buffered substrate, sodium-beta-glycerophosphate and monosodium diethylbarbiturate, and 2 c.c. of cerebrospinal fluid were incubated at 37° C. for twenty-four hours in a small rubber-stoppered flask. At the end of this time the flask was cooled, 8 c.c. of 5 per cent trichloracetic acid were added, and the mixture was shaken and filtered. To 5 c.c.

of this filtrate were added 4 c.c. of molybdate reagent and 1 c.c. of stannous chloride solution. The blue color developed was read in a colorimeter against the color developed on similar treatment with molybdate and stannous chloride by a standard phosphate solution containing 0.004 mg. per c.c. The difference in milligrams of phosphorus found after incubation and that initially present in the cerebrospinal fluid was taken as the measure of phosphatase activity.

Lipase was determined by a slight modification of the method of Crandall and Cherry,[13] which depends on the determination by titration with sodium hydroxide of the free acid formed by the enzymatic hydrolysis of olive oil. A mixture containing 3 c.c. of distilled water, 2 c.c. of a 50 per cent olive oil emulsion made up with 5 per cent gum acacia as the emulsifying agent, 0.5 c.c. of Clark and Lubs' buffer at pH 7.0, and 1 c.c. of cerebrospinal fluid was incubated at 37° C. for twenty-four hours and then titrated with N/20 sodium hydroxide. Since it was found that some hydrolysis of the olive oil took place even though no cerebrospinal fluid was present in the above mixture, a blank was run using 1 c.c. of water instead of cerebrospinal fluid. The lipolytic activity, expressed in millimoles of fatty acid formed in twenty-four hours per 100 c.c. of cerebrospinal fluid, is:

$$\text{Lipase units} = (\text{c.c. NaOH}_{CSF} - \text{c.c. NaOH}_{H_2O}) \times 5 \times 100.$$

The tributyrin and ethyl butyrate-splitting power of the cerebrospinal fluid was determined by the above method, replacing the 2 c.c. of olive oil emulsion with 1 c.c. of absolute tributyrin or 1 c.c. of absolute ethyl butyrate. The calculations for tributyrinase and esterase were the same as those for lipase.

The amylolytic activity of the cerebrospinal fluid was determined by a modification of the method of Ross and Shaw,[14] which depends on the hydrolysis of starch or glycogen by the enzymatic material and the oxidation of the resulting reducing sugars (considered as maltose) by hypoiodite. To a mixture of 25 c.c of freshly prepared 1 per cent starch solution, 10 c.c. of phosphate buffer at pH 6.8, 1 c.c. of 0.2 N sodium chloride, and 3 c.c. of distilled water, was added 1 c.c. of cerebrospinal fluid. The resulting mixture was shaken and 10 c.c. were pipetted into a small Erlenmeyer flask containing 0.5 c.c. of N hydrochloric acid. The acid and phosphate buffer were neutralized with N sodium hydroxide. Then 2 c.c. of 0.1 N iodine solution and 3 c.c. of 0.1 N sodium hydroxide were added and the mixture allowed to stand for fifteen minutes. One cubic centimeter of N hydrochloric acid was added to liberate the excess iodine, which was titrated with 0.02 N sodium thiosulfate. After twenty-four hours, 10 c.c. of the hydrolysis mixture were withdrawn and treated similarly. The measure of amylolytic activity was taken as:

$$\text{Amylase units} = \text{millimoles of maltose formed in 24 hours per 100 c.c. of cerebrospinal fluid}$$
$$= (\underset{\text{after 0 hours}}{Na_2S_2O_3 \text{ titer in c.c.}} - \underset{\text{after 24 hours}}{Na_2S_2O_3 \text{ titer in c.c.}}) \times 4 \times 100,$$

since 1 c.c. of 0.02 N $Na_2S_2O_3$ = 3.41 mg. maltose = 0.01 millimoles maltose.

TABLE I

NORMAL CEREBROSPINAL FLUID

CASE	DIAGNOSIS	CELL COUNT	TRYPSIN	ANTI-TRYPSIN UNITS	PHOSPHATASE	LIPASE	TRIBUTYRINASE	ESTERASE	AMYLASE	TOTAL PROTEIN	SUGAR	CHLORIDES	NON-PROTEIN NITROGEN
			mM. Tyrosine		*Mg. Phosphorus*	*mM. Fatty Acid*			*mM. Maltose*	*Mg. Per Cent*			
B. B.	Epilepsy	1	0	0	0.4	0	0	0	0.8	19	54	730	13
R. P.	Epilepsy	3	0	0	0	0.4	0	0	2.0	14	58	712	
J. B.	Epilepsy	4	0	0		0		0	0	25	58	687	14
W. S.	Epilepsy	3	0	0	0.9	0.8	0.8	0	0.6	7	75	735	
J. A.	Epilepsy	3	0	0		0		0	1.0				
L. N.	Epilepsy	6	0	0	0.4	0.9	0	0	1.2	3	50	715	20
L. B.	Epilepsy	0	0			0	0	0	2.0	46	56	748	14
N. W.	Epilepsy	0	0			0	0	0	1.8	5	45	710	12
M. M.	Cerebral birth trauma	8	0	8	0.2	0.3	0	0	0	13	47	718	17
D. Ma.	Cerebral birth trauma	12	0	0	0.3	0	0	0	0	17	67	731	
J. S. (1)	Cerebral birth trauma	0	0			0	0	0					
J. S. (2)	Cerebral birth trauma	7	0	9	1.8	0	0	0	0.6	8	53	702	18
C. H. (1)	Cerebral birth trauma	2	0	20				0	3.3				
C. H. (2)	Cerebral birth trauma	3	0	0		0.8		0	0	31		721	17
A. F. (1)	Cerebral birth trauma	4	0	0		0		0	1.0	31			8
A. F. (2)	Cerebral birth trauma	2	0			0	0	0		13			10
N. J.	Cerebral agenesis	4	0	0	1.6	0	0	0	0.7	26	50	702	10
M. K. (1)	Cerebral agenesis	7	0	20		0	0		0.8		61		
M. K. (2)	Cerebral agenesis	0	0	0	1.4	0	0	0	0				
J. Be.	Cerebral agenesis	5	0	0		0	0	0	1.7	Pandy –	62	729	17
S. S.	Idiocy	4	0						2.8	28	45	710	18
F. L.	Idiocy	3	0	0		0	0	0	1.0				
D. R.	Idiocy	6	0	0	1.8	0	0	0	0			730	
M. F.	Idiocy		0	0	1.9	0	0	0	0				18
B. A. F.	Idiocy		0	0				0	0				
B. Ba.	Idiocy	3	0	0	0.4	0	0		0.6				
D. D.	Idiocy	3	0	18	0.7	0	0	0	0.4	18	65	723	

Glycogenase was determined by the same method as amylase, except that 0.5 per cent glycogen was used as a substrate instead of starch. In this case it was necessary to use 3 drops of 1 per cent starch solution as an indicator for the thiosulfate titration.

Sugar was determined by the method of Somogyi;[15, 16] chlorides by the method of Van Slyke;[17] total protein by the method of Ayer, Dailey and Fremont-Smith;[18] nonprotein nitrogen by the method of Koch and McMeekin.[19] Total proteins were also done by a digestion method which was checked against the above-mentioned method for total protein.

RESULTS

For convenience in describing and discussing our results, we have divided the fluids studied into several groups.

Group I: Normal.—The fact that a spinal puncture is considered necessary indicates that the patient is not entirely normal, as has been pointed out previously.[1] However, as can be seen from Tables I and VII, the cell counts and sugar, chlorides, total protein, and nonprotein nitrogen contents of these fluids were normal.[1, 9, 20-22] Though not reported in this paper, the calcium and phosphorus contents of these fluids were also found to be normal. The enzyme distribution in these specimens was, therefore, considered to be that of normal cerebrospinal fluid.

None of the 27 fluids of the normal group showed any evidence of tryptic activity. Of 22 fluids studied 18 had no antitryptic properties; the remaining 4 fluids had slight antitryptic power. Phosphatase was found in 12 of 13 fluids, with an average activity of 0.9 unit (see methods for explanation of the units used throughout the paper). The actual values of the phosphatase activity ranged from 0.2 to 1.9 units. Lipolytic activity was found in small amounts in 4 fluids of 24 studied. The average lipolytic activity of the 24 fluids was negligible, being within the experimental error. Tributyrinase was found in a small amount, 0.8 unit, in only 1 of 20 fluids studied. Esterase was not found in any of the 24 fluids studied. Amylase was present in 17 of 25 fluids of group I, the average amylolytic activity of the 25 fluids being 0.9 unit. The individual activities of the 17 fluids which gave positive results varied from 0.4 to 3.3 units. Discussion of glycogenase is omitted since in all cases where glycogenase activities were determined the results paralleled those of amylase.

It appears from these results that beta-glycerophosphatase and amylase are present in detectable quantities in an appreciable number of cerebrospinal fluids with normal chemical and cellular findings. Trypsin and esterase were not found. Lipase, tributyrinase, and antitrypsin were found in small amounts in relatively small percentages of the fluids studied.

Group II: Tuberculous Meningitis.—In this group there were 23 fluids obtained from 13 patients. In general, the cellular and chemical constituents were in accord with the literature. In one case there were a low cell count and also a normal chloride content. This was due to the fact that the fluid was drawn during the early stage of the meningitis which followed a tuberculoma. The same explanation covers the normal sugar content in another case.

TABLE II

TUBERCULOUS MENINGITIS

CASE	APPEARANCE OF FLUID	CELL COUNT	TRYPSIN	ANTI-TRYPSIN UNITS	PHOSPHATASE	LIPASE	TRIBUTYRINASE	ESTERASE	AMYLASE	TOTAL PROTEIN	SUGAR	CHLORIDES	NONPROTEIN NITROGEN
			mM. Tyrosine		*Mg. Phosphorus*	*mM. Fatty Acid*			*mM. Maltose*	*Mg. Per cent*			
C. W.	Clear	150	0.05	21		0	0	0	2.4	Pandy +	16	648	
*H. W. (1)	Clear	19	0.04	26		0	0.9	0	0	275	13	724	14
H. W. (2)	Clear	65	0.06	70		0	1.5	0	1.4	Pandy +			
Le. W. (1)	Clear	220	0.17	0		0	0	0	1.4	Pandy +	27	679	
Le. W. (2)	Pink (Prontosil)	160	0.12	16	1.8	0	0	0	1.8	Pandy +	24	700	
Le. W. (3)	Pink (Prontosil)	175	0.11	45		0	0.7	0	1.0	Pandy +	20	700	
L. S. (1)	Clear	450	0.04	0	1.3					Pandy +	40		
L. S. (2)	Clear	450	0.23	6	2.9	0.5			0	Pandy +	36		
L. S. (3)	Clear	590	0.13	88	4.7	2.4	3.2			700	16		26
R. C.	Opalescent	350	0.04	19	1.1	1.1	1.3	0	1.6	67	26	722	
E. T.	Clear		0.04	0	0.9					Pandy +			
G. R. (1)	Clear	175	0.08	35	1.7	0	0.7	0	0	210	13	605	19
G. R. (2)	Opalescent	180	0.16	47	1.8	0.8	2.5	0		174	10		
G. R. (3)	Opalescent	850	0.30	80	3.2	0	0.8	0		235	10		
G. R. (4)	Opalescent	700	0.08	50	2.8	0.5	0.8	0	3.6	200		569	
C. F.	Clear	98	0.04	23	2.6	1.3	2.7	0	4.2	171	10	656	
R. N.	Turbid		0.29	0					2.8	Pandy + +			
L. C.	Clear	190	0.08	19		0.5		0	5.5	50		649	22
M. W. (1)	Opalescent	124	0.10	0		0		0	1.8	106			6
M. W. (2)	Opalescent		0.10	9		1.6		0	1.0	69	17	587	14
I. W. (1)	Clear	126	0	25	1.7	0	0.4	0	2.6	83	27	605	
I. W. (2)	Clear		0.03	47	3.1	0	0.8	0	1.4	105	30	675	
†R. M. P.	Clear	160	0		0.9	0	0	0		Pandy +	28		

*Tuberculoma of the brain followed by meningitis.
†Diagnosis unconfirmed.

TABLE III

PURULENT MENINGITIS

CASE	DIAGNOSIS	APPEARANCE OF FLUID	CELL COUNT	TRYPSIN	ANTI-TRYPSIN UNITS	PHOS-PHATASE	LIPASE	TRIBU-TYRINASE	ESTERASE	AMYLASE	TOTAL PROTEIN	SUGAR	CHLO-RIDES	NON-PRO-TEIN NITRO-GEN
				mM. Tyrosine		*Mg. Phos-phorus*	*mM. Fatty Acid*			*mM. Maltose*	*Mg. Per cent*			
A. McQ. (1)	Influenzal	Turbid	5000	0.08	91		0.7	2.0		0		10	673	
A. McQ. (2)	Influenzal	Turbid	7200	0.65	44		0	0	0	0				
A. McQ. (3)	Influenzal	Turbid	11000	1.71	80		0	1.9	0.9	2.2		10		
A. McQ. (4)	Influenzal	Turbid	2000	1.20	8		4.3	0	0	1.6		10		
R. W.	Influenzal	Cisternal turbid	5000	1.44	34		0	1.4	1.0	0		10	681	
R. H.	Influenzal	Cisternal turbid		0.86	17		0		0	2.4	481	10	669	21
W. G. (1)	Streptococcic (anhemolyticus) and	Cisternal turbid	600	0.26	0		0	0	0	0	38	10		24
W. G. (2)		Cisternal turbid	4600	1.40	0	9.0	0							
W. G. (3)	Staphylococcic (albus)	Ventricular turbid	5000	1.80	0	22.0	1.7	3.3	2.9	2.8	942			
G. O. (1)	Streptococcic (hemolytic and viridans)	Ventricular turbid	8500	0.71	4		0		0	0.8		18	732	
G. O. (2)		Ventricular turbid	720	0.18	64			0	0	2.6	Pandy ++++	10	650	
G. O. (3)	Streptococcic (hemolytic and viridans)	Ventricular turbid	900	0.80	61		0	0	0	2.3	Pandy ++++			
J. D. (1)	Streptococcic (hemolytic)	Ventricular turbid	2290	0.53	39		0		0	0	81			13
J. D. (2)	Streptococcic (hemolytic)	Opalescent	440	0.20			0				32			18
J. B.	Staphylococcic	Turbid	6000	1.30	10	22.0					375			
J. J.	Pneumococcic	Turbid	5000	1.60	66	14.0	0	1.7			89	27*	758*	
D. R. (1)	Meningococcic	Turbid	3000	0.67	7	14.4	2.5	2.2	0.6	0	61	58*	736*	
D. R. (2)	Meningococcic	Turbid	1200	0.40	0	8.0	1.3	3.4	0.9	0.8				

*These values are of doubtful significance because of the large amounts of intravenous glucose and saline which the patient received during treatment.

On the whole the cerebrospinal fluid of tuberculous meningitis was characterized by increased enzymatic activity. Most striking was the presence of trypsin and of increased amounts of antitrypsin. Twenty-one of 23 fluids had definite tryptic activity, ranging from 0.03 to 0.30 unit, with an average of 0.10 unit. Antitrypsin was found to be present in 16 of 22 fluids studied, with values for the antitryptic power from 6 to 88 units, and an average of 28 units for all the fluids. Phosphatase activity, studied in 14 fluids of this group, was found to be about twice as great as that of the normal fluids. Values from 0.9 to 4.7 units were found, with an average value of 2.1 units. In 8 of 20 fluids studied for lipolytic activity, values as high as 2.4 units were encountered; the average activity was 0.3 unit. Tributyrinase was present in 12 of 16 fluids, with activities as high as 3.2 units and an average of 1.0 unit. Esterase was not found in any of the 18 fluids tested for this enzyme. Amylase was present in 14 of 17 fluids studied. The average activity was 1.9 units, with a maximum value of 5.5 units.

Group III: Purulent Meningitis.—The results of the chemical studies agreed with those of the literature. The total protein contents were elevated, with values as high as 940 mg. per cent. Sugars and chlorides were nearly always reduced. White cell counts were markedly elevated, with values from 440 to 11,000 per c. mm., and a predominance of polymorphonuclear leucocytes.

In general, the distribution of enzymes in the fluids of the purulent meningitides was similar to that in tuberculous meningitis, except that far larger amounts of trypsin and phosphatase were present in purulent meningitis. All the 18 fluids studied contained trypsin, in amounts ranging from 0.08 unit to 1.80 units, with an average value of 0.87 unit. Fourteen fluids studied showed antitryptic power, varying from 4 to 91 units, with an average value of 31 units. Phosphatase activity was measured in only 6 fluids, but these were consistently high in phosphatase activity, ranging in value from 8 to 22 units, with an average value of 15 units. With one exception no higher value for phosphatase activity was found in any of the fluids studied in the whole series. Lipase was present in 6 of 16 fluids studied; its presence appeared to depend on the severity of the disease. Tributyrinase was found in 7 of 12 fluids, with activities as high as 3.4 units and an average of 1.3 units. Esterase appeared in some fluids in the advance stages of the disease. Amylase was present in 8 of 14 fluid studies, with an average value of 1.1 units.

Group IV: Hydrocephalus.—Nearly all of the fluids of this group presented a different enzyme picture from that of the normal fluids. The extent of the difference depended on the cause of the hydrocephalus and in some cases on the presence of infection. For convenience we shall distinguish between two subgroups of fluids. The first subgroup consisted of five fluids all of which were abnormal in appearance, being opalescent, xanthochromic, or turbid. In one patient, G. C., the hydrocephalus was found on autopsy to have been caused by occlusion of the foramen of Monro by an old hemorrhage into the third ventricle. In another patient, W. W., the hydrocephalus was

caused by a cerebellar medulloblastoma which invaded the fourth ventricle. The other three fluids of this subgroup were from two patients with hydrocephalus complicated by infection and showed definite leucocytosis. The protein contents of the 5 fluids were high, ranging in value from 456 to 2500 mg. per cent.

The sugar content of the infected fluids was zero; all of the noninfected fluids were normal. The chloride values were not significantly diminished. All the fluids had marked antitryptic power and were high in lipase, tributyrinase, and phosphatase. One fluid, that of R. K., had a very high tryptic activity; two fluids with a relatively low polymorphonuclear content had low trypsin values, while the noninfected fluids were normal with respect to trypsin. Amylase values were normal, and only one fluid, that of R. K., showed esterase activity.

The fluids of the second subgroup, 13 in number, had relatively lower protein contents, the values ranging from 5 to 103 per cent. Eleven of the fluids were clear; one was contaminated by a few red cells, and one fluid was xanthochromic. Seven fluids had tributyrinase activity, with values from 0.7 to 3.0 units. Ten fluids had lipolytic activity, with values from 0.6 to 7.7 units. With respect to the other enzymes and antitrypsin the fluids of this subgroup showed no significant variations from the normal.

On the whole, therefore, fluids from patients with hydrocephalus where there was an associated lesion invading a ventricle, or which were complicated by infection, had greater enzymatic activity than fluids from patients in whom there was no known involvement of meninges or ventricles.

Group V: Brain Tumor, Abscess, Cyst.—The fluids of this group were similar to those of Group IV in that they could be divided conveniently into two subgroups with greater or less enzymatic activity, according to whether or not there was a lesion invading a ventricle or the meninges. The first subgroup includes one fluid from a patient with a medulloblastoma of the cerebellum invading the fourth ventricle (Table V, W. W.), four fluids from a patient with a cerebral subarachnoid cyst (R. McC.), and one fluid from a patient with an abscess of the right parietal lobe (C. K.). In the last case the abscess broke into the subarachnoid space so that the fluid was turbid, like that of a suppurative meningitis. The three cases of this subgroup have in common the fact that the lesions of the brain invaded spaces containing cerebrospinal fluid, viz., fourth ventricle and subarachnoid space. Of the five fluids making up the second subgroup, 2 were from a patient (E. P.) with a glioblastoma of the middle fossa, two from a patient (L. C.) with a cerebral abscess of undetermined location, and one from a patient with an intracerebellar glioma.

Of the 6 fluids of the first subgroup, 5 (W. W. and R. McC.) were xanthochromic and had highly elevated protein contents (416 to 660 mg. per cent); the sixth (C. K.) had a total protein content of 103 mg. per cent. The fluids of the second subgroup were clear, with protein contents ranging from 58 to 207 per cent. The average values of the chloride and sugar contents for all the fluids of group V fell within the ranges of normal values, with no significant individual variations.

TABLE IV

HYDROCEPHALUS

CASE	APPEARANCE OF FLUID	CELL COUNT	TRYPSIN	ANTI-TRYPSIN UNITS	PHOS-PHATASE	LIPASE	TRIBU-TYRINASE	ESTERASE	AMYLASE	TOTAL PROTEIN	SUGAR	CHLO-RIDES	N.P.N.	COMMENT
			mM. Tyrosine		*Mg. Phos-phorus*	*mM. Fatty Acid*			*mM. Maltose*	*Mg. Per Cent*				
First Subgroup														
W. H. (1)	Opalescent, ventricular	310 (Poly.)	0.04	65	6.9	1.0	2.1	0	0	680	0	646	–	Staph. aureus pyocephalus, spina bifida
W. H. (2)	Opalescent, ventricular	350 (Poly.)	0.03	34	14.2	1.1	4.3		1.7	650	0	694	0	
R. K.	Turbid, ventricular	Frank pus	2.32	26	66.4	1.1	7.4	3.9	0	2500	0			B. pyocyaneus pyocephalus, spina bifida
G. C.	Xanthochromic, ventricular		0	83		4.3		0	1.0	456			15	Occlusion of foramen of Munro by hemorrhage into choroid plexus
W. W.	Xanthochromic, lumbar	40 Lymph.	0	93	5.8	0.5	2.5	0	0.7	613	33	686	20	Medulloblastoma of cerebellum with invasion of the fourth ventricle

TABLE IV—CONT'D

Second Subgroup

B. S. (1)	Clear, ventricular		0	0	0.6	3.0	1.4	0	0	41	43	679		
B. S. (2a)	Clear, lumbar	30 Lymph.	0	6	3.1	0.9	0.7	0	2.6	103	39	696		
B. S. (2b)	Clear, ventricular	10 Lymph.	0	6	0.8	0.6	2.4	0	3.6	46	36	654		
M. R. (1)	Clear, lumbar	20 Lymph.	0	0	1.0	0.8	3.0	0	0	16	49	738		
M. R. (2)	Clear, lumbar	1 Lymph.	0	6	1.1	0.4	1.3	0	0	17	53	724		
J. M.	Clear, ventricular	3 Lymph.	0	0	0.8	5.4	2.3	0	0	13	93*	890*		Glioma of cerebellum without invasion of meninges or ventricle
R. G. (1)	Clear, lumbar	2	0			0.6	0	0	0			748		
R. G. (2)	Clear, lumbar	2	0	0	0.7	0	0	0	0	13	58	745	18	
N. L. W.	Clear, ventricular		0	5	1.6	0.8	0	0	0	5	36	763	24	Spina bifida
B. G. B. (1)	Clear, ventricular		0	0		2.0		0	0.9	53			20	Spina bifida
B. G. B. (2)	Clear, ventricular		0	0		0	0.8	0	1.2	72		716	22	
B. G. B. (3)	Bloody, ventricular		0	0		0	0	0	0.6					
B. B. L.	Xanthochromic, ventricular		0.9	0		7.7		0	0	69				

*These high values are due to intravenous injection of glucose in saline.

TABLE V

BRAIN TUMOR, BRAIN ABSCESS, BRAIN CYST

CASE	DIAGNOSIS	APPEARANCE OF FLUID	CELL COUNT	TRYPSIN	ANTI-TRYPSIN UNITS	PHOSPHATASE	LIPASE	TRIBUTYRINASE	ESTERASE	AMYLASE	TOTAL PROTEIN	SUGAR	CHLORIDES	NON-PROTEIN NITROGEN
				mM. Tyrosine		*Mg. Phosphorus*	*mM. Fatty Acid*			*mM. Maltose*	*Mg. Per cent*			
First Subgroup														
W. W.	Cerebellar medulloblastoma	Xanthochromic	40 Lymphs.	0	93	5.8	0.5	2.5	0	0.7	613	33	686	20
R. McC. (1)	Cerebral subarachnoid cyst	Xanthochromic	0	0	34		1.7		0	3.8	463	49	711	14
R. McC. (2)	Cerebral subarachnoid cyst	Xanthochromic	110 Lymphs.	0.48	69		0	1.3	0.6	0.9	416		722	15
R. McC. (3)	Cerebral subarachnoid cyst	Xanthochromic		0	28	2.0	3.0	2.8		1.0				
R. McC. (4)	Cerebral subarachnoid cyst	Xanthochromic		0	88	7.7	0	3.0	0.5	1.8	660	53	814	15
C. K.	Cerebral abscess	Turbid	3000 Polys.	1.40	38	11.4	1.0	5.8		7.8	103	56	710	
Second Subgroup														
E. P. (1)	Glioblastoma of middle fossa	Clear	120 Lymphs.	0	0	0.8	0.4	3.8			207			
E. P. (2)	Glioblastoma of middle fossa	Clear	150 Lymphs.	0	0	1.0	1.2	1.8	0	0	154	29	685	
L. C. (1)	Cerebral abscess	Clear	170 Lymphs.	0.15	7	1.0	1.0	0.4			58			
L. C. (2)	Cerebral abscess	Clear	22 Lymphs.	0.04	18	0.7	0	0.7			80	63		
J. M.	Cerebellar glioma	Clear (ventricular)	3 Lymphs.	0	0	0.8	5.4	2.3	0	0	13	93	890	

The fluids of the first subgroup possessed marked enzymatic properties. Two fluids of the 6 had high tryptic activities, 0.47 and 1.4 units, respectively. All 6 fluids possessed antitryptic power, with values ranging from 28 to 93 units, and an average value of 58 units. Two fluids of this subgroup (W. W. and C. K.), examined for phosphatase activity, had values of 5.8 and 11.4 units, respectively. Four of the 6 fluids had lipolytic activity, with values from 0.5 to 3.0 units, and an average of 1.0 unit for the whole subgroup. Five fluids examined for tributyrinase activity gave positive results, with values from 1.3 to 5.8 units, and an average of 3.1 units. Of 4 fluids examined for esterase activity, 2 were found to have activities of 0.6 and 0.5 units; the other 2 had no esterase activity. The amylolytic activity of 6 fluids ranged from 0.7 to 7.8 units. The last value, obtained in patient C. K., was the highest amylase activity found in the entire study.

Of the second subgroup, 3 fluids (E. P. and J. M.) were similar to normal fluids with respect to trypsin, antitrypsin, phosphatase, esterase, and amylase. All 3 fluids, however, had lipolytic activity, 0.4, 1.2, and 5.4 units, and tributyrinase activity (3.8, 1.8, and 2.3 units). The other 2 fluids (L. C.) had some tryptic activity (0.15 and 0.04 unit), some antitryptic power (7 and 18 units), and slight lipase and tributyrinase activities. The phosphatase was normal. On the whole, the fluids of this subgroup differed considerably less from the normal than those of the first subgroup.

Group VI: Miscellaneous.—This group contains all fluids studied which did not fall into any of the five groups so far discussed. It includes fluids from patients with acute benign lymphocytic meningitis (2), poliomyelitis (3), and encephalitis (4), as well as one patient with cerebral arteriosclerosis, one patient with hemihypertrophy of the body, 3 patients with chorea, one patient with suspected carbon monoxide poisoning, one patient with postencephalitic syndrome, one patient with postdiphtheritic tabes, and one patient with multiple sclerosis.

Increased cell counts (lymphocytes) were found in the majority of these fluids (Table VI). Protein contents were elevated in 5 fluids of the 12 studied. The concentrations of chlorides and sugar were normal in all fluids studied. Despite the occurrence of increased cell counts and elevated protein contents in some cases, the enzyme findings in the fluids of this group were normal for the most part. One patient with polioencephalitis (D. M.) showed deviations from the normal. Positive, though small, tryptic activity and antitryptic power were found; high tributyrinase activity was present, and phosphatase was slightly increased. Abnormal lipolytic activity was found in 2 fluids. The values found were 9.0 and 5.4 units, respectively. Four other fluids had lipolytic activities somewhat higher than the normal.

DISCUSSION

The present work has shown that the normal cerebrospinal fluid contains beta-glycerophosphatase and in many cases amylase, but that other enzymes such as trypsin, lipase, tributyrinase, and esterase are either entirely absent, or are present in small quantities in relatively few instances. The latter results are in agreement with those of Heyde[23] and Hiller[3] who studied

TABLE VI

MISCELLANEOUS

CASE	DIAGNOSIS	CELL COUNT	TRYPSIN	ANTI-TRYPSIN UNITS	PHOSPHATASE	LIPASE	TRIBUTYRINASE	ESTERASE	AMYLASE	TOTAL PROTEIN	SUGAR	CHLORIDES	NON-PROTEIN NITROGEN
			mM. Tyrosine		*Mg. Phosphorus*	*mM. Fatty Acid*			*mM. Maltose*	*Mg. Per cent*			
C. W.	Cerebral arteriosclerosis	4	0	0	0.8	1.7	0	0	2.5	15	63	694	15
H. K.	Hemihypertrophy of the body	3	0	0	0.6	1.2	0	0	1.0	11	56	713	
C. Wo.	Huntington's chorea	10	0	0		0			2.0	31	63	696	
H. W.	Chorea	4	0	0	1.7	0	0	0	0.8				
A. M. B.	Chorea	6	0	0	0.7	0	0	0	0				
S. S.	Carbon monoxide poisoning	1	0	0		5.4		0	0	Pandy +	73	728	15
L. M.	Postencephalitic syndrome	12	0	0	0.9	0	0	0	0	56	57	734	13
T. W.	Postdiphtheritic tabes	8	0			0		0	0	56		741	23
E. Z.	Multiple sclerosis	15	0	0		9.0		0	0	Pandy +	52	734	
B. M.	Acute benign lymphocytic meningitis	350	0	0		0		0	1.8		87		
E. I.	Acute benign lymphocytic meningitis	280	0	0	1.1								
P. A. H.	Preparalytic poliomyelitis	93	0	0		0	0	0		42			
L. T.	Preparalytic poliomyelitis	120	0	14	0.6	1.1			0		49		
D. M.	Polioencephalitis?	9	0.03	10	2.2	0.9	2.9	0	1.8	155			
K. B.	Mumps meningoencephalitis	170	0	12		0.4		0	3.0	44			15
L. W.	Postpneumonic encephalitis		0	0	2.0	0	0	0	1.6				
A. W.*	Miliary tuberculosis	200	0	7	0.4	0	0			11			
C. T.	Typhoid	80	0		0.2	1.1	0.4			6			

*On autopsy no meningitis was found.

proteolytic and lipolytic enzymes in normal cerebrospinal fluid and in fluids from patients with schizophrenia, senile dementia, and epilepsy. In tuberculous meningitis, purulent meningitis, hydrocephalus, brain tumor, brain abscess, and brain cyst, the cerebrospinal fluid is characterized by increase in the enzymatic activity. The question arises as to the source of the cerebrospinal fluid enzymes, both in normal and pathologic conditions of the central nervous system. Some of the possibilities will be briefly discussed.

Since enzymes are generally considered to be proteins, the problem of explaining the presence in normal cerebrospinal fluid or phosphatase and amylase is closely related to the still unsolved problem of the source of the other proteins of the cerebrospinal fluid. Some authors[9] hold that under normal conditions proteins do not pass from the blood into the cerebrospinal fluid but originate within the cerebrospinal cavity. There is also some evidence to show that the cerebrospinal fluid acts not only as a mechanical protector of the cerebrospinal system, but also as a medium for the interchange of metabolic material between the nervous tissue and the blood system. This interchange includes the removal of waste products from the nervous tissue. Investigation of the enzymes of normal brain tissue has indicated that this tissue contains appreciable amounts of phosphatase[24] and small amounts of amylase,[25, 26] but very little, if any, proteolytic or lipolytic enzymes.[3, 27-29] Oxidases which have been found in brain tissue in considerable quantities[30, 31] have also been reported in normal cerebrospinal fluid. There is, therefore, some parallelism between the enzymes of normal brain tissue and those of the normal cerebrospinal fluid. This may lend support to the hypothesis that the cerebrospinal fluid takes an active part in the metabolism of the central nervous system. The proteins and enzymes of the normal cerebrospinal fluid, according to this theory, may be liberated into the fluid during the normal metabolism of brain tissue.

On the other hand, it may be said that since the blood contains phosphatase and amylase, the enzymes of the normal cerebrospinal fluid may be derived from the blood. If this were the case, one would naturally expect to find tributyrinase, lipase, esterase, and antitrypsin in the cerebrospinal fluid, since these substances are also present in the blood. In order to account for the very small concentrations in which these substances are found in the cerebrospinal fluid, or for their absence, it would be necessary to assume that the degree of permeability of the meninges and plexi differs normally for the various enzymes. There is at present insufficient experimental basis for a definite decision on this point. In connection with this problem it is interesting to note that cholinesterase has been reported present both in brain tissue and in blood, but not in the cerebrospinal fluid.[32] It may be that the cerebrospinal fluid contains cholinesterase-inhibiting substances analogous to the antitrypsin of the blood serum, or that the nervous tissue-fluid and blood-fluid barriers are such that they do not permit the passage of cholinesterase into the cerebrospinal fluid. M. B. Bender has recently reported (Am. J. Physiol. **126**: 180, 1939) that using the facial nerve of the cat as an indicator, he found cholinesterase activity in the cerebrospinal fluid equivalent to 1 to 2 per cent of that of blood serum.

The source of the enzymes of the cerebrospinal fluid in meningitis, both tuberculous and purulent, is also unsettled as yet. The possible sources are (a) increased permeability of the meninges, which may allow passage from the blood; (b) the liberation of enzymes by the destruction of brain tissue; and (c) the increase in the number of leucocytes in the fluid. It is difficult to determine quantitatively the relative importance of these sources. The known increased meningeal permeability in meningitis,[8] coupled with the fact that phosphatase, tributyrinase, lipase, and antitrypsin are found in greatly increased amount in the cerebrospinal fluid of meningitis, tends to show that these substances come at least in part from the blood. The occurrence of some breakdown of brain tissue in meningitis[33] would also help to explain the increased phosphatase found in meningitis. It is questionable whether this breakdown would give rise to any noticeable amounts of proteolytic or lipolytic enzymes because of the scarcity of such enzymes in brain tissue. The presence of a tryptic enzyme in the cerebrospinal fluid of meningitis is explainable, therefore, neither on the grounds of increased meningeal permeability nor in terms of destruction of brain tissue. In the purulent meningitides, where the polymorphonuclear leucocytes often amount to 98 per cent of all the cells of the fluid, the average trypsin and phosphatase activities were found to be about nine times as great as in tuberculous meningitis where the cell counts were lower and where the polymorphonuclear cells constituted only about 10 to 20 per cent of the cells. Since it has been shown[34, 35] that the polymorphonuclear cells are much richer in trypsin than the lymphocytes, the presence of trypsin in the cerebrospinal fluid of meningitis can probably be attributed to the polymorphonuclear cells. These cells have also been shown to have considerable phosphatase activity,[36] so that the high phosphatase activity of the fluid in purulent meningitis can be attributed in part to the same cells. Finally, since the antitrypsin, lipase, and tributyrinase values were quite similar in tuberculous and in purulent meningitis, but the trypsin and phosphatase values were much higher in purulent meningitis, we believe that the polymorphonuclear cells are chiefly responsible for the trypsin and phosphatase, while increased meningeal permeability is chiefly responsible for the antitrypsin, lipase, and tributyrinase.

Our work has shown that the enzymes in the cerebrospinal fluid of hydrocephalus, brain tumor, brain abscess, and brain cyst differ in general from those found in normal cerebrospinal fluid and in the fluids of meningitis. Thus it was also necessary to divide the specimens of group IV and group V into subgroups, according to whether or not the meninges or ventricles were invaded, either by a neoplasm or by hemorrhage. However, this division provides a clue to the source of the enzymes. It has been found[8] that brain tumors which invade the meninges or ventricles cause an increase in meningeal permeability, while tumors which do not affect the meninges or ventricles have no influence on meningeal permeability. The fluids of groups IV and V which had high enzymatic activity were obtained from patients with the following pathologic conditions: hydrocephalus due to occlusion of the foramen of Monro by a hemorrhage into the choroid plexus, hydrocephalus caused by a cerebellar medulloblastoma invading the fourth ventricle, pyo-

cephalus, cerebral subarachnoid cyst, and a cerebral abscess which broke into the subarachnoid space. In all of these cases meninges or ventricles were invaded, so that there was probably increased meningeal permeability. The fact that the phosphatase, lipase, tributyrinase, and antitrypsin values were high in these fluids indicates that their high enzymatic activity was due in considerable measure to the increase in meningeal permeability. In one case (cerebral abscess which broke into the subarachnoid space) the fluid was very turbid and had a high polymorphonuclear count. The trypsin and phosphatase activities of this fluid were particularly high, in agreement with the idea that these enzymes, when present in appreciable quantities, are derived from the polymorphonuclear cells. In 3 patients of group V, a glioblastoma of the middle fossa, an intracerebellar glioma, and a cerebral abscess of undetermined location, the enzymatic activity was less than that of the patients in whom the meninges or ventricles were known to be involved. The same was true in 4 patients with relatively mild hydrocephalus. Three of these patients are alive 6 to 8 months after the studies were made; the fourth was a recent study. In some of these fluids, however, an interesting question is raised by the presence of high lipase and tributyrinase activities (Table IV, B. S., B. G. B., J. M., M. R., B. B. L.; Table V, E. P., L. C.). In these cases it is unlikely that the meningeal permeability is sufficiently increased to account for the presence of these enzymes. The leucocyte contents of these fluids are also unable to account for the high lipase and tributyrinase activities found. It is, therefore, possible that the presence of these enzymes is in some way associated with destruction of brain tissue. It is known[37, 38] that in nerve degeneration, myelin is converted into droplets of unsaturated fats which are further broken down and disappear. The presence in brain tissue of large amounts of myelin and other lipoids, together with the occurrence of tissue degeneration in pathologic conditions of the brain such as tumor, abscess, sclerosis, and encephalitis, make it appear reasonable to assume that the presence of lipase and tributyrinase in the cerebrospinal fluid is associated with the degeneration of brain tissue. It is possible that the high lipolytic activity found in isolated cases of multiple sclerosis, cerebral arteriosclerosis, and carbon monoxide poisoning, may be explained on this basis. Whether the enzymes in question are part of the cause of the degeneration or appear as one of the effects is a question which cannot be answered as yet.

Another point of interest is brought out by our data on lipase, tributyrinase, and esterase. It can be seen from Tables I to VI that the ability of the cerebrospinal fluid to hydrolyze olive oil does not parallel its ability to hydrolyze tributyrin or ethyl butyrate. In 15 pathologic fluids both lipase and tributyrinase were found; in 15 others, tributyrinase was present but not lipase; in 5 fluids, lipase was present but not tributyrinase. Eleven fluids in which tributyrinase activity was not determined showed lipolytic activity. Since esterase was found in only 5 fluids, there is no parallelism between the occurrence of lipase, tributyrinase, and esterase. Of the 5 fluids in which esterase was found, all had tributyrinase activity, but only one had lipolytic activity. It appears from this that it is necessary to distinguish carefully

TABLE VII

	GROUP I NORMAL (EPILEPSY AND MENTAL DEFICIENCY)				GROUP II TUBERCULOUS MENINGITIS				GROUP III PURULENT MENINGITIS				GROUP IV HYDROCEPHALUS				GROUP V BRAIN TUMOR, BRAIN CYST, BRAIN ABSCESS			
	NO. OF FLUIDS	MIN.	MAX.	AVG.	NO. OF FLUIDS	MIN.	MAX.	AVG.	NO. OF FLUIDS	MIN.	MAX.	AVG.	NO. OF FLUIDS	MIN.	MAX.	AVG.	NO. OF FLUIDS	MIN.	MAX.	AVG.
Enzymes (Expressed in units per 100 c.c. of fluid)																				
Trypsin mM. tyrosine	7	0	0	0	23	0	0.3	0.10	18	0.08	1.7	0.87	*5	0	2.32		6	0	1.4	
													13	0	0.9	0.01	5	0	0.15	
Antitrypsin units	22	0	20	3	22	0	88	28	16	0	91	31	5	26	93	60	6	28	93	58
													12	0	7	3	5	0	18	
Phosphatase Mg. P	13	0	1.9	0.9	14	0.9	4.7	2.2	6	9.0	22	15	4	5.8	66.4		2	5.8	11.4	8.6
													8	0.6	3.1	1.2	5	0.7	1.0	0.9
Lipase mM. fatty acids	34	0	0.9	0	20	0	2.4	0.4	16	0	4.3	0.7	5	0.5	4.3		6	0	3.0	1.0
													13	0	7.7		5	0	5.4	1.6
Tributyrinase mM. fatty acid	20	0	0.8	0	16	0	3.2	1.0	12	0	3.4	1.3	4	2.1	7.4		5	1.3	5.8	3.1
													11	0	3.0		5	0.4	3.8	1.8
Esterase mM. fatty acid	24	0	0	0	18	0	0	0	13	0	2.9	0.5	5	0	3.9		4	0	0.6	0.3
													13	0	0	0	2	0	0	
Amylase mM. maltose	25	0	3.3	0.9	7	0	5.5	1.9	14	0	3.8	1.1	5	0.7	1.7		6	0.7	7.8	
													13	0	3.6		2	0		
Chemical Constituents (Expressed in mg. per 100 c.c. of fluid)																				
Total protein	16	5	46	21	12	50	275	145	8	32	941	262	5	456	2500		5	103	660	450
													11	5	103		5	13	207	125
Sugar	15	45	75	56	17	0	40	21	8	0	18	10	4	0	33		4	33	56	48
													7	36	93		3	29	93	
Chlorides	16	687	748	719	13	569	724	656	5	650	732	681	3	646	694		5	686	814	729
													10	654	890		2	685	890	
Nonprotein nitrogen	14	8	20	15	6	14	26	17	4	13	24	19	2	15	20	18	4	14	20	16
													4	18	21	19				

*In groups IV and V the upper number refers to the first subgroup, the lower number to the second subgroup.

between the ability of the cerebrospinal fluid to hydrolyze true fats, simple esters, and esters of glycerol and low molecular weight fatty acids. The failure to do so has resulted in some confusion in the literature, since the terms "lipase" and "esterase" have both been used for the tributyrin-splitting enzyme.

Another question which arises in connection with our work is the possibility of utilizing determinations of the enzymes of the cerebrospinal fluid in diagnosis of diseases of the central nervous system. Since the enzymatic properties of the cerebrospinal fluid in tuberculous and purulent meningitis differ from those of the normal fluid, the enzymes may be of use in diagnosis. Also, the fluid of tuberculous meningitis may be distinguished from that of purulent meningitis by means of the trypsin and phosphatase activities. Remembering that fluids from cases of the preparalytic stage of poliomyelitis and from cases of encephalitis and acute benign lymphocytic meningitis were practically normal, it may be hoped that the determination of enzymes may aid in the differential diagnosis of these diseases from tuberculous meningitis. Although it is at present too early to make more definite statements on this subject, enzyme studies have been found to be of some help in our laboratories and further work is at present in progress.

The study of hydrocephalus fluid shows that the clinical diagnosis of hydrocephalus alone, without knowledge of the cause of the condition, leads to a very limited understanding of the chemical and enzymatic findings. Even the division of hydrocephalus into such classes as communicating internal, obstructive internal, and external hydrocephalus indicates little of the etiology and course of the condition. It is necessary whenever possible to find the cause of the hydrocephalus. Our results indicate that it may be possible to distinguish on the basis of the enzymatic properties of the cerebrospinal fluid between cases where the meninges or ventricles are invaded by a neoplasm or hemorrhage, and cases where the hydrocephalus is due to congenital malformation other than a neoplasm.

Similarly, enzyme determinations may give some knowledge of the location of tumors, in particular whether or not they invade the ventricles or meninges. Finally, further work may indicate a closer relationship between the presence of lipase and tributyrinase in the cerebrospinal fluid and the extent of fatty degeneration of brain tissue.

CONCLUSIONS

1. Comparative studies have been made of the enzymes, cells, and chemical constituents of 113 specimens of normal and pathologic cerebrospinal fluid from 77 patients. The fluids were examined for trypsin, antitrypsin, phosphatase, lipase, tributyrinase, esterase, and amylase, as well as for cellular contents, sugar, chlorides, total protein and nonprotein nitrogen.

2. Normal cerebrospinal fluid was found to contain small amounts of beta-glycerophosphatase and amylase, and occasional traces of lipase, tributyrinase, and antitrypsin. Trypsin and esterase were never found in these fluids.

3. The cerebrospinal fluid of tuberculous meningitis had increased enzymatic activity in addition to abnormal chemical findings and elevated cell

counts. Trypsin occurred in practically all fluids from patients with tuberculous meningitis. Antitrypsin and tributyrinase were each found in about 75 per cent of the fluids. Phosphatase was found in all these fluids, generally elevated above the normal. Lipase was found in an appreciable number of fluids; esterase was never found. Amylase was elevated above the normal.

4. The cerebrospinal fluid of purulent meningitis contained trypsin and phosphatase in much greater amounts than did the fluid of tuberculous meningitis. Values for antitrypsin, tributyrinase, and lipase were similar to those found in tuberculous meninges. Esterase was found in a few fluids. Amylase was practically normal.

5. The cerebrospinal fluid of our hydrocephalus patients fell into two groups, depending on whether or not there was a lesion of the brain which invaded the meninges or ventricles. The fluids of the former group were characterized by xanthochromia, high protein content, lipolytic activity, high antitryptic power, elevated phosphatase and tributyrinase activities. The fluids of the latter group were normal, except for the marked lipolytic activity found in some cases.

6. The cerebrospinal fluid of our patients with brain tumor, abscess, and brain cyst, also fell into two groups, depending on whether or not the lesion invaded the meninges or ventricles. The fluids of the former group were found to have generally increased enzymatic activity and antitryptic power. The fluids of the latter group showed less deviation from the normal enzyme distribution, with occasional trypsin, antitrypsin, lipase, and tributyrinase.

7. In a group of patients with miscellaneous diagnoses, the fluids were normal, except for a patient with suspected polioencephalitis and isolated cases of multiple sclerosis, carbon monoxide poisoning, and cerebral arteriosclerosis. In the last three cases, high lipolytic activity was found.

8. The possible sources of the enzyme found in normal and pathologic cerebrospinal fluids are discussed. It is suggested that the enzymes of the normal cerebrospinal fluid may result from the metabolism of brain tissue or may be derived from the enzymes of the blood. Increased enzymatic activity of pathologic cerebrospinal fluid may be due to (a) increased meningeal permeability, (b) increased number of leucocytes, or (c) destruction of brain tissue.

9. The increased lipase and tributyrinase present in the cerebrospinal fluid in pathologic conditions not resulting in increased meningeal permeability may be associated with degeneration of brain tissue.

10. The possible application of enzyme studies in the diagnosis of tuberculous meningitis, hydrocephalus, brain tumor, brain abscess, and brain cyst is considered.

REFERENCES

1. Levinson, A.: Cerebrospinal Fluid in Health and in Disease, St. Louis, 1929, C. V. Mosby Company.
2. Kafka, V.: Die Zerebrospinalflüssigkeit, Leipzig and Vienna, 1930, F. Deuticke.
3. Hiller, F.: Die Beziehungen der degenerativen Veränderungen des Zentralnervensystems zu seinem Gehalt an Fett und Esterspaltenden Enzymen; sowie der Nachweis sowohl dieser Enzyme als auch von Lipoidstoffwechselschlacken des Nervengewebes im Liquor Cerebrospinalis, Ztschr. f. d. ges. Neurol. u. Psychiat. **109:** 263, 1927.

4. Marchionini, A., and Ottenstein, B.: Diastaseuntersuchungen bei Neurosyphilis, Deutsche Ztschr. f. Nervenh. **128:** 83, 1932.
5. Dochez, R. A.: Proteolytic Enzymes and Anti-enzymes of Normal and Pathological Cerebrospinal Fluids, J. Exper. Med. **11:** 718, 1909.
6. Szabo, J.: Über Fermentwirkungen des Liquor Cerebrospinalis bei verschiedenen Geisteskrankheiten, Ztschr. f. d. ges. Neurol. u. Psychiat. **17:** 145, 1913.
7. Flexner, L. B.: The Chemistry and Nature of the Cerebrospinal Fluid, Physiol. Rev. **14:** 161, 1934.
8. Walter, F. K.: Die Blut-Liquor Schranke, Leipzig, 1929, Georg Thieme.
9. Katzenellenbogen, S.: The Cerebrospinal Fluid and Its Relation to the Blood, Baltimore, 1935, Johns Hopkins Press.
10. Anson, M. L., and Mirsky, A. E.: The Estimation of Trypsin With Hemoglobin, J. Gen. Physiol. **17:** 151, 1933.
11. Hecht, M., and Civin, H.: Studies on Enzyme Action. I. The Estimation of Pepsin and Trypsin in Yeast, J. Biol. Chem. **116:** 477, 1934.
12. Bodanksy, A.: Phosphatase Studies. II. Determination of Serum Phosphatase. Factors Influencing the Accuracy of the Determination, J. Biol. Chem. **101:** 93, 1933.
13. Crandall, L. A., and Cherry, I. S.: The Specificity of Pancreatic Lipase. Its Appearance in the Blood After Pancreatic Injury, Am. J. Physiol. **100:** 266, 1932.
14. Ross, J. R., and Shaw, M. M.: The Effect of Dehydration on the Pancreatic and Intestinal Enzymes, J. Biol. Chem. **104:** 131, 1934.
15. Somogyi, M.: Notes on Sugar Determination, J. Biol. Chem. **70:** 599, 1926.
16. Somogyi, M.: A Method for the Preparation of Blood Filtrates for the Determination of Sugar, J. Biol. Chem. **86:** 655, 1930.
17. Van Slyke, D. D.: The Determination of Chlorides in Blood and Tissues, J. Biol. Chem. **58:** 523, 1923.
18. Ayer, J. B., Dailey, M. E., and Fremont-Smith, F.: Denis-Ayer Method for the Quantitative Estimation of Protein in the Cerebrospinal Fluid, Arch. Neurol. & Psychiat. **26:** 1038, 1931.
19. Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. **46:** 2066, 1924.
20. Merritt, H. H., and Fremont-Smith, F.: The Cerebrospinal Fluid, Philadelphia and London, 1937, W. B. Saunders Company.
21. Levinson, A., and Cohn, D.: Comparative Dextrose Content of Lumbar and Cisternal Cerebrospinal Fluid, Am. J. Dis. Child. **51:** 17, 1936.
22. Cohn, D. J., Levinson, A., and McCarthy, F.: Physiological Variations in the Glucose Rate of Blood and Cerebrospinal Fluid, Am. J. Physiol. **103:** 613, 1933.
23. Heyde, W.: Zur Kenntnis der Proteasen der Cerebrospinalflüssigkeit, Ztschr. f. d. ges. Neurol. u. Psychiat. **138:** 536, 1932.
24. Giri, K. V., and Datta, N. C.: Brain Phosphatase, Biochem. J. **30:** 1089, 1936.
25. Takasaka, T.: Über den Fermentgehalt des Menschlichen Gehirns, Biochem. Ztschr. **184:** 390, 1927.
26. Wohlgemuth, J., and Scesci, S.: Zur Kenntnis von der Entstehung und Zusammensetzung der Zerebrospinalflüssigkeit, Ztschr. f. d. ges. Neurol. u. Psychiat. **13:** 455, 1912.
27. Edelbacher, S., Goldschmidt, E., and Schlappe, V.: Über die Enzyme des Gehirns, Ztschr. f. Physiol. Chem. **227:** 118, 1934.
28. Blum, E., Jakovtschuk, A., and Jarmoschkewitsch, A.: Die proteolytische Ferments des Gehirns, Bull. Biol. Med. exp. U. S. S. R. **1:** 17, 1936; Physiol. Abst. **21:** 778, 1936.
29. Krebs, H. A.: Über die Proteolyse der Tumoren, Biochem. Ztschr. **238:** 174, 1931.
30. Gerard, R. W.: Metabolism of Brain and Nerve, Ann. Rev. Biochem. **6:** 419, 1937.
31. Cohen, M. B., and Gerard, R. W.: Oxidizing Enzymes in Brain Extracts, Am. J. Physiol. **119:** 34, 1937.
32. Stedman, E., and Stedman, E.: The Relative Choline-Esterase Activities of Serum and Corpuscles From the Blood of Certain Species, Biochem. J. **29:** 2107, 1935.
33. Levinson, A.: Meningitis Masked by Encephalitis Symptoms, Arch. Pediat. **49:** 6, 1932.
34. Opie, E. L.: Intracellular Digestion, Physiol. Rev. **2:** 552, 1922.
35. Wilstatter, R., Bamann, E., and Rhodewald, M.: Zur Kenntnis der proteolytischen Wirkung farbloser Blutkorperchen, Ztschr. f. physiol. Chem. **185:** 267, 1929.
36. Fiessinger, N., and Boyer, F.: Les Phosphatases des Leucocytes, Enzymologia **1:** 172, 1936.
37. Karsner, H. T.: Human Pathology, Philadelphia, 1935, J. B. Lippincott Company.
38. Weil, A.: A Textbook of Neuropathology, Philadelphia, 1933, Lea & Febiger.

NORMAL HEMATOLOGIC STANDARDS IN THE AGED*

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HEMATOLOGIC standards have been made for the ages from infancy to thirty years; studies of the blood for ages beyond thirty years, and especially for the older age groups (past sixty years), have been very few and meager. Blood studies are important in both medical and surgical conditions. Before we can report variations from the normal blood count, we must know the normal values for the various ages and sex. We are presenting a hematologic study of 160 men over sixty years of age, who were inmates of a home for the aged.

Nascher[1] quoted the normal blood determinations in the aged as: red blood cells—3,000,000 to 5,500,000; white blood cells—4,000 to 8,000; hemoglobin—90 to 110 per cent. Leichtenstern[2] in 1878 found a large drop in hemoglobin from 55 to 60 years, and a rise in old age. Williamson[3] disagreed with Leichtenstern's findings; he stated that the report was based on too few cases. He studied the hemoglobin of 919 persons, of whom 152 (81 males, 71 females) were over 60 years. He found that the values were fairly level from the ages of 16 to 60 years (16.9 gm. hemoglobin per 100 c.c. of blood in men). Then the values declined to the age of 75 years (15.22 gm. hemoglobin); after 76 years there was a small rise (15.67 gm. hemoglobin). The values for the women past 60 years were less than for the men, but the differences were not as great as at the younger ages. Osgood[4] made hematologic determinations on various age groups, but his grouping did not include older men and women; in the male group, 14 to 30 years, the average red blood cell count was 5,400,000; hemoglobin, 15.8 gm. He felt that Williamson's values were accurate in relation to each other but were all too high. Wintrobe,[5] in his studies of erythrocyte counts and hemoglobin, which included mostly adults from 18 to 30 years and a small group of older people, concluded that there are no significant differences due to old age. He showed the erythrocyte counts, hemoglobin, and volume of packed red blood cells in men 30 to 60 years and in men over 60 years of age to be approximately the same as those of younger men. He stated that no significant differences occurred in normal adults as age advanced as long as health remained good. Price-Jones[6] found that an increased cell diameter in the aged was usually associated with some degree of emphysema.

Studies of the older age groups vary with the examiner and the country. Williamson,[3] in Chicago, found an average hemoglobin of 15.81 gm. in 81 men over 60 years. Rud,[7] in Denmark, found an average hemoglobin of 13.66 gm. and red blood cell count of 5,140,000 in 7 men over 60 years. Bing,[8] also in

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Denmark, found an average red blood cell count of 6,100,000 in 22 men over 60 years. In a study of 50 men over 60 years in Brussels, Millet and Balle-Helaers[9] determined the average hemoglobin to be 13.3 gm., and the red cell count, 3,900,000. Bing suggested a deficient cardiac power or local changes in the skin and capillaries as possible causes for his high red blood cell counts, since hypertension was not present in his patients. Millet and Balle-Helaers' counts were low. Various forms of anemia may have been present in some of their patients.

TABLE I

AVERAGE HEMATOLOGIC RESULTS BY FIVE-YEAR GROUPS IN 160 MEN

AGES	NO. OF MEN	HB.	R.B.C.	W.B.C.	STAFF %	NEUTROPHILE %	LYMPHOCYTE %	MONOCYTE %	EOSINOPHILE %	BASOPHILE %
60-64	13	15.3	4.74	8,842	1	56	32	7	3	0.3
65-69	36	14.6	4.49	7,742	1	58	30	8	3	0.5
70-74	39	13.7	4.32	7,752	1.5	58	30	7	2	1
75-79	36	14.2	4.44	7,114	1.5	58	29	8	2.5	0.5
80-84	26	14.4	4.51	8,032	1	55	31	9	3	1
85-89	7	14	4.64	8,042	2	55	31	9	2	1
94-104	3	14.1	4.23	10,816	0	61	27	11	1	0
Averages		14.3	4.46	7,709	1.3	57.5	30	7.8	2.7	0.7

TABLE II

HEMATOLOGIC STANDARDS IN OLD AGE AND A COMPARISON WITH OSGOOD'S STANDARDS IN MALES, 14 TO 30 YEARS

	IN MALES—60 TO 104 YEARS		OSGOOD'S STANDARDS[4] IN MALES —14 TO 30 YEARS	
	AVERAGE VALUE	RANGE OF VALUES IN 95% OF CASES	AVERAGE VALUE	RANGE OF VALUES IN 95% OF CASES
Hemoglobin in grams	14.3	12-17.5	15.8	14-18
R.B.C. in millions	4.46	3.5-5.5	5.4	4.6-6.2
W.B.C.	7,700	4,000-13,000	7,400	4,500-11,500
Staff cells per cent	1.3	0-4	0.8	0-5
Neutrophiles per cent	57.5	40-75	54	33-78
Lymphocytes per cent	30	15-50	38	18-65
Monocytes per cent	7.8	1-15	4	0-9
Eosinophiles per cent	2.7	0-8	1.9	0-6
Basophiles per cent	0.7	0-2	0.5	0-2

Our studies included red and white blood cell determinations, hemoglobin, and a differential smear on 160 men, with ages ranging from 60 to 104 years. In all these patients blood was obtained from the finger pulp. Our red and white blood cell counts were done with standard pipettes and a Levy-Hausser counting chamber. Hayem's solution was used for the red blood cell and Turck's solution for the white blood cell counts. The hemoglobin determinations were made with the Hellige wedge hemometer. Our differential smears were stained with Wright's solution and 200 cells were counted on each slide. These determinations were all made in the summer months (June, July, and August) and in the late afternoon.

Table I shows the average hematologic results by five-year groups. Table II gives our average hematologic values for the 60 year and over group, and

presents the range of values in over 95 per cent of these patients. We also compared our average findings with those of Osgood[4] which he obtained in his younger males.

DISCUSSION

In the older age groups, senile retrogressive changes, arteriosclerosis, pulmonary emphysema, and hypertension are present to a greater or lesser degree. The degenerative changes are an integral part of the senescent process, and when we speak of normal old men we include those with changes which are peculiar to their age and exclude those with inflammatory or neoplastic pathologic processes. In our present study we included ambulatory patients who were institutionalized for custodial care and whose physical examination was negative except for changes incidental to old age.

The average red blood cell count in our series was 4,460,000 per c.mm., with a range from 3,500,000 to 5,500,000. Both Osgood and Wintrobe in their younger subjects found an average red blood cell count of 5,400,000, with a range of values from 4,600,000 to 6,200,000. The average red blood cell count in the aged is 81 per cent of the average found in the younger ages; in other words, the count per c.mm. is one million less for the older age groups than for the normal adults. Hemoglobin diminishes proportionately to the decrease in red blood cells. We found an average hemoglobin of 14.3 gm. per 100 c.c. of blood, with a range from 12 to 17.5 gm. Osgood found an average hemoglobin of 15.8 gm., and Wintrobe, of 16 gm.; their range of values was 14 to 18 gm. There was an average decrease of 1.5 to 1.7 gm. of hemoglobin per 100 c.c. of blood in comparing the determinations in the young and the old. On the basis of a 5,000,000 red blood cell count the value for 100 per cent hemoglobin in our study was 16 gm. With Osgood's figures the equivalent of 100 per cent hemoglobin is 14.7 gm. Wintrobe suggested the use of 14.5 gm. hemoglobin as 100 per cent.

TABLE III

HEMOGLOBIN AND THE ERYTHROCYTES IN RELATION TO BLOOD PRESSURE

	NO. OF MEN	HEMOGLOBIN IN GRAMS	RED BLOOD CELLS IN MILLIONS
Hypotensive cases	6	14.3	4.37
Normal blood pressure cases	83	14.3	4.50
Hypertensive cases (140-160)	17	14.4	4.43
Hypertensive cases (160 and over)	54	14.2	4.43

An increase in red blood cells occurs in people living in high altitudes, in chronic pulmonary diseases, and in myocardial insufficiency. The altitude of the institution where our blood studies were made is 300 feet above sea level. This elevation is not sufficient to affect our results. We omitted from this study all patients with cardiac murmurs, dependent edema, dyspnea, cyanosis, pulmonary fibrosis, and pulmonary tuberculosis. We included hypertensive patients. As Table III indicates, we divided our patients into three main groups according to their blood pressure. The normal blood pressure group included those with systolic pressures between 120 and 140 and diastolic pressures of 90 or under. The hypertensive group included those

with systolic pressures over 140 and diastolic pressures over 90. In the hypotensive group were those with systolic pressures under 120. The hypertensive group was subdivided into those with systolic pressures between 140 and 160 and those with systolic pressures of 160 and over. The normal blood pressure group had the highest red blood cell count (4,500,000), and the hypotensive group, the lowest (4,370,000). The 140 to 160 blood pressure group had the highest hemoglobin average (14.4 gm.), while the 160 and over group had the lowest (14.2 gm.). The differences in the red blood cell count and in the amount of hemoglobin in the various groups were slight. This finding differs from the results obtained by Doles[10] who reported an increase in the red blood cell count with hypertension.

We can only surmise the causes of anemia in old age. Chronic low grade infections, not sufficient to alter the white blood cell count, may be a factor. In a study of blood sedimentation rates in the aged,[11] we have shown that one-third have a sedimentation rate above normal. Oral sepsis is prevalent, pyorrhea and carious teeth being the main causes. Prostatic hypertrophy is common in this age group. This produces an obstruction to the urinary outflow and stagnation of urine in the urinary bladder, with an increased possibility of infection and urinary decomposition. Bronchiectasis may also play a part in this picture. Taylor and Schwartz,[12] in a roentgenologic study of 125 apparently normal men from this institution, found interstitial changes suggesting bronchiectasis in 36 cases (27 per cent). The stagnant bronchial secretion may be a fertile soil for chronic sepsis. Nephrosclerosis, the common kidney finding in old age, may also play a part in the production of the anemia. Decrease in the cellularity of the red bone marrow with advancing age may be another factor.

The average leucocyte count was 7,700 cells, with a range from 4,000 to 13,000. Osgood, in his younger group, found an average of 7,400 cells, ranging from 4,500 to 11,500. The minimum and average counts agreed fairly well, but the old age group showed a higher maximum value.

The differential smear gave average percentages within normal limits. A comparison of our findings with those of Osgood is presented in Table II; the average percentages and the range of values for the various white blood cells are shown. Thirty-three of our patients had an eosinophile count of 5 per cent and over. The highest was 16 per cent. We believe that this was due to the skin changes that occur in old age. Atrophy, seborrhea, dryness, and pruritus are common dermatologic findings in the aged.

CONCLUSIONS

1. The red blood cells per cubic millimeter of blood are diminished in old age. The average red blood cell count is 4,460,000. Chronic low grade infection, nephrosclerosis, and decreased cellularity of the red bone marrow in old age may be causative factors. Hypertension did not produce an increase in the red blood cells.

2. The hemoglobin per 100 c.c. of blood is decreased in the aged, the average being 14.3 gm. The decrease in hemoglobin is proportional to the decrease in red blood cells.

3. The white blood cells are within normal limits.
4. Differential counts are within normal limits.

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REFERENCES

1. Nascher, I. L.: Geriatrics, The Diseases of Old Age and Their Treatment, Philadelphia, 1914, P. Blakiston's Son & Co.
2. Leichtenstern: Quoted by Williamson, C. S.: Influence of Age and Sex on Hemoglobin, Arch. Int. Med. **18**: 505, 1916.
3. Williamson, C. S.: Influence of Age and Sex on Hemoglobin, Arch. Int. Med. **18**: 505, 1916.
4. Osgood, E. E.: Normal Hematologic Standards, Arch. Int. Med. **56**: 849, 1935.
5. Wintrobe, M. M.: The Erythrocyte in Man, Medicine **9**: 195, 1930; Blood of Normal Men and Women, Bull. Johns Hopkins Hosp. **53**: 118, 1933.
6. Price-Jones, C.: The Signs of Red Blood Cells in Emphysema, J. Path. & Bact. **24**: 326, 1921.
7. Rud, E. J.: Red Corpuscles and Their Variations, Acta med. Scandinav. **57**: 142, 1922; **57**: 325, 1922.
8. Bing, H. I.: Number of Red Blood Corpuscles at Different Ages and Under Different Conditions, Acta med. Scandinav. **53**: 833, 1921.
9. Millet and Balle-Helaers, E.: Hemoglobin Values in the Aged, Sang. **6**: 735, 1932.
10. Doles, H. M.: The Erythrocyte and Its Relation to Blood Pressure, Virginia M. Monthly **62**: 489, 1935.
11. Miller, I.: Blood Sedimentation Rates in Middle-Aged and Old People, J. Lab. & Clin. Med. **21**: 1227, 1936.
12. Taylor, H. K., and Schwartz, S.: Roentgen Findings of the Thorax in the Aged, Quart. Bull., Sea View Hosp. **3**: 250, 1938.

LABORATORY METHODS

A SHORT CUT IN GAS ANALYSIS*

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IN CURRENT methods of gas analysis where more than one gas must be estimated, only the first gas dealt with can be absorbed in one unbroken series of visits to its appropriate absorption pipette. In the case of each gas subsequently estimated, the "dead space"† in each of the absorption pipettes previously visited must be flushed out several times (usually three separate times) for the complete elimination of that gas.

Suppose, for example, that certain gases a, b, and c, in *II* of Fig. 1, are to be removed in order from a gas sample measured in burette *Bt* by visits to absorption pipettes *A*, *B*, and *C*, respectively. The procedure would run as follows:

1. The gas sample, after initial measurement of volume, is driven over into *A* the requisite number of times, say N_1, and the reduced volume read.

2. Next the gas is driven into *B* a certain number of times, say N_2, then returned to *A* to flush out the dead space, driven back to *B* for another series of visits, N_3, returned to *A* twice more with subsequent series of visits, N_4 and N_5, to *B*. The reduced volume is then read on returning to the graduated burette.

3. The gas must now be driven over into *C* with correspondingly more complicated interruptions for the purpose of revisiting *A* and *B* to remove the last traces of the gas c. This involves a series of interrupted visits N_6, N_7, N_8, N_9 to *C*, with flushings of the dead spaces in *A* and *B* in between each constituent series of these visits. Thus to complete the analysis some nine series of trips to absorbing fluids must be made, together with several flushings of capillary spaces.

Quite apart from the loss of time due to interrupting the visits to an absorption pipette, the total number of trips involved is much greater than that necessary to completely absorb that constituent from one undivided sample of gas. Thus if 15 trips to the pyro solution were necessary to remove quantitatively the oxygen from a gas sample driven without interruption into and out of *C*, then not less than 35 to 40 total visits would be required for this purpose in the routine analysis to ensure complete absorption of the oxygen from the flushings of the dead spaces in *A* and *B*.

If the burette in question has a capacity of about 10 mils it does not in general require a great many trips to pyro to remove adequately the oxygen,

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†By the "dead space" is meant the space in each absorption pipette *A*, *B*, *C* above the level of the fluid meniscus, *m*. In the pipettes II of Fig. 1 it also includes the volume of the oblique bore of the stopcock leading to the pipette in question.

but if, on the other hand, sufficiently accurate analyses are required to demand the use of a pipette with a capacity of 20 to 25 mils, the complete absorption of oxygen from this larger gas sample requires a more than proportionate number of trips to pyro. It was just such a tedious procedure requiring the estimation of carbon dioxide, acetylene, and oxygen from a gas sample that led to this attempt to shorten the process.

The simplest possible procedure in such an analysis would be realized if the absorption of each gas could be accomplished by one unbroken series of visits to its appropriate pipette. This could be achieved *if each constituent of the gas mixture could be absorbed from all but an accurately known small fraction of the total gas sample*. This could in turn be performed if the following conditions could be fulfilled.

(1) That an accurately known amount of the gas sample could be separated from the rest and left behind in the dead space of any given pipette after the absorption of one gas in it was complete.

(2) If subsequently either (a) the whole of any unabsorbed gas remaining there in the first place was finally left within that pipette until the next analysis or (b) a completely negligible amount of any unabsorbed gas remaining there in the first place was finally left within that pipette until the next analysis.

By an appropriate redesigning of the standard Haldane and Haldane-Grollman apparatus, these conditions were completely and quantitatively fulfilled. In the case of the acetylene fraction of the gas sample conditions 1 and 2 (b) were fulfilled, and in the case of the oxygen fraction conditions 1 and 2 (a). In an alternative method of using the apparatus conditions 1 and 2 (a) can be fulfilled in the case of both these gases.

Detail of the Changed Design.—In Fig. 1, *I* represents the general design of the standard Haldane apparatus, *A* being the pipette for the absorption of carbon dioxide and *B* the pipette for the absorption of oxygen. The type of three-way tap above *A* and *B* is shown in cross section enlarged below (*III*). In Fig. 1, *II* represents the Haldane-Grollman apparatus with pipettes *A* for carbon dioxide, *B* for acetylene, and *C* for oxygen. The type of three-way tap above *A*, *B* and *C*, is shown in *IV* in longitudinal section.

In the first type of apparatus the dead space is equal to about 1/50 of the total gas sample, and in the second type it is still more, due to the additional length of horizontal capillary which is the inevitable result of using the oblique stopcock. This dead space could be reduced in either type of apparatus by setting the menisci higher by means of the adjustable clamp usually employed with such apparatus. The reduction so effected, however, would still leave too large a dead space with the caliber of tubing currently used, and, moreover, with the oblique stopcock the smallest capillary tubing that could be employed for use with both gas and liquid would still leave that residual volume too great. (The horizontal length of capillary cannot be reduced to zero.)

Since it is highly desirable to employ the oblique type of stopcock in preference to the three-way type, as much less liable to develop leaks with extended use, this disadvantage of excessive dead space was obviated by designing a stop-

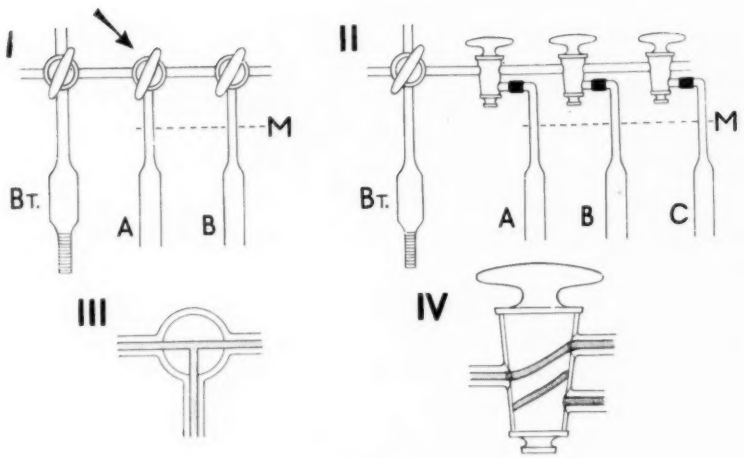

Fig. 1.

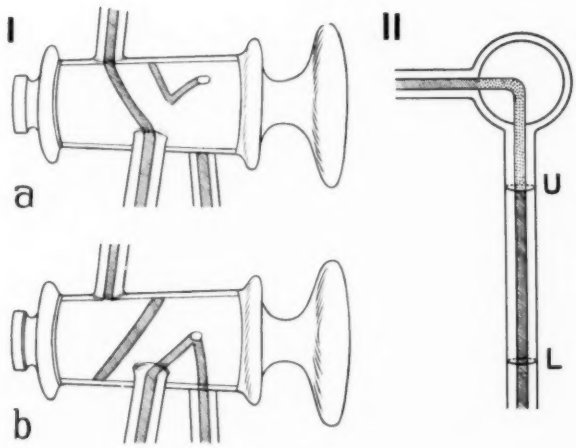

Fig. 2.

cock to combine the separate advantages of the two types shown. This is illustrated in Fig. 2, *I*, which was drawn from photographs of such a stopcock taken *from the direction* shown by the arrow in Fig. 1. In *b* the tap is turned 180° from the position *a*. This stopcock may be described as a modification of the ordinary oblique tap, produced by accomplishing the imaginary act of bending one of the parallel bores at right angles at the center. This permits making a vertical lead out tube coming straight down from the stopcock and avoiding the waste horizontal length shown in Fig. 1, *II*. Further, by reducing the bore of the capillary, the residual dead space (remaining when the visits to a pipette are completed and the liquid meniscus finally adjusted) was cut down to an extremely small volume shown by the stippled space in *II* of Fig. 2. This, of course, includes the right angled bore of the stopcock.

By varying the capillary bore in different experimental pipettes, an optimum diameter was finally secured which was sufficiently small for the purpose

in hand and yet sufficiently large for use with fluids. In a similar way, starting with a stopcock drilled with too fine a bore, and involving difficulties both with regard to undue resistance to the flow of gas and a tendency to become partially blocked with tap grease, this bore was enlarged by drilling several times, until a diameter compatible with all desiderata was reached.

In the case of the lead out capillary (*UL* of Fig. 2) an optimum diameter was found to be about 1.5 mm., while the corresponding dimensions in the case of the stopcock were finalized at about 1.8 mm. diameter for the straight bore and about 1.7 mm. for the right angled bore.

Detail of the Analytical Procedure (Procedure A).—The following description is given with reference to the general layout of Fig. 1, *I*. The reader is asked to imagine that the stopcocks shown here are replaced by the type illustrated in Fig. 2, *I*, and that Fig. 2, *II*, represents the capillary joining each absorption pipette to the stopcock above it. After the absorption of the carbon dioxide in pipette *A* and measurement of the changed volume with the meniscus set at the lower level *L*, the adjusting screw is used to raise the fluid level to the upper mark *U*, just a few millimeters below the stopcock. This stopcock is then turned to the position shown in *I a* of Fig. 2, and the gas driven into and out of pipette *B* a sufficient number of times (previously determined) to completely absorb the acetylene. The menisci of *B* and *A* are then set to their corresponding *L* marks* and the second reduced volume is read in the burette.

Next, before proceeding to drive the gas into *A* the fluid levels in *A* and *B* are both raised to the *U* levels and the stopcocks are appropriately turned. When sufficient visits to pyro have been made, the final *procedure must be carried out rigidly as follows:*

1. The pyro meniscus is set to its mark.

2. The stopcock is turned to communicate with *B* and the bulk of the nitrogen driven into that pipette to dilute the contents of the residual dead space and so reduce the percentage of oxygen to a negligible amount.

3. The fluid meniscus is set to the *L* level in *B* and the same procedure as 2 repeated in the case of pipette *A*.

4. The final volume is read in the burette and the uncorrected percentages of carbon dioxide, acetylene, and oxygen are calculated.

5. The acetylene percentage is then increased† by 1/500 and the oxygen percentage by 1/250 (corresponding exactly in each case to the volume of gas locked up in the residual dead spaces), and the analysis is complete.

The following example illustrates in the order of the maximum corrections involved, since the percentage amount of oxygen in the samples analyzed seldom exceeds 22 per cent. The uncorrected analysis gave 5.37 per cent carbon dioxide, 10.54 per cent acetylene, and 22.19 per cent oxygen.

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*The setting of the meniscus of the capillary attached to the control burette is assumed at each reading of the burette, but omitted from the text to simplify the description.

†These convenient ratios did not fall out by chance when the apparatus was constructed. They were altered several times as a consequence of redrilling the stopcocks to secure the optimal bore. The correction concerned was then about 1/440 for each pipette. By using fine wire rings around the capillary for graduation marks, exact positions were found to yield the more suitable ratios quoted above, and so permit of calculation by immediate inspection. The method for accurately determining the volumes of the residual dead spaces is described in the *Addendum.*

The correction for the acetylene amounts to 1/500 of 10.54 per cent, i.e., 0.021 per cent or 0.02 per cent to the nearest significant figure, while the correction for the oxygen amounts to 1/250 of 22.19 per cent, i.e., 0.088 per cent or 0.09 per cent to the nearest significant figure.

Further, since the total volume of the capillary system of the manifold is less than 1/50 of the burette volume, the effect of the final flushing procedure is to remove all except 1/50 of these correction residuals. Inasmuch as this final residue has a maximum value of 1/50 of 0.09 per cent, it will be obviously quite negligible in respect to the succeeding analysis.

To illustrate the accuracy of the method a series of five consecutive pairs of duplicate analyses, occurring in routine estimation of the cardiac output by Grollman's acetylene method, are given in Table I. The duplicate analyses were made by two separate machines.

TABLE I*

	MACHINE A	MACHINE B
Carbon dioxide	6.48	6.48
Acetylene	8.62	8.63
Oxygen	15.35	15.37
Total	30.45	30.48
Carbon dioxide	5.67	5.65
Acetylene	9.59	9.60
Oxygen	16.65	16.66
Total	31.91	31.91
Carbon dioxide	4.94	4.93
Acetylene	10.38	10.39
Oxygen	17.46	17.47
Total	32.78	32.78
Carbon dioxide	6.75	6.76
Acetylene	8.25	8.25
Oxygen	14.64	14.64
Total	29.64	29.64
Carbon dioxide	5.57	5.57
Acetylene	7.06	7.05
Oxygen	16.42	16.44
Total	29.05	29.06

*It should be noted by those interested in analyses of acetylene that the degree of accuracy illustrated in Table I is not obtainable unless the mercury in the burette has been thoroughly cleaned. When the mercury surface becomes dirty, the analytical accuracy falls off remarkably. This has been observed by others but no complete explanation has yet been given. The series in the table has, therefore, been selected from this point of view, but it is a fair representation of the accuracy of which the method is capable.

Further Simplification of the Analysis (Procedure B).—When a series of analyses are being made a simplification of the foregoing procedure may be adopted with advantage. In this the final flushing of the absorbing pipettes with nitrogen may be omitted without loss of accuracy provided the following routine be strictly observed.

1. After the pyro meniscus has been set, the stopcock is turned to pipette *B*, when the elevated liquid column immediately drops. This is quickly pushed down to the lower engraved mark by means of the adjusting screw, the meniscus

is set, and the stopcock turned off to an oblique position. The effect of this procedure is to carry the oxygen within the residual dead space down to the lower portion of the gross dead space and to prevent effectually its diffusion back into the capillary of the manifold. The same procedure is then repeated in the case of pipette A. In this way these two small oxygen fractions are kept within the system, and become absorbed during the next analysis. Since the error thereby introduced is only 1/250 of the difference in the percentages of oxygen in the two samples, it is clear that no correction need be applied unless the difference in composition amounts to about 3 per cent. This seldom happens in a series of analyses. If under exceptional conditions a greater difference in two samples does occur, it is merely a matter of a moment's inspection to make an accurate correction. Thus if an analysis yielding 20 per cent of oxygen followed another showing 14 per cent of oxygen, the former would require a correction of 1/250 (20-14), i.e., 0.02 per cent. In order to test the accuracy of the procedure just described (Procedure B), an experimental mixture of carbon dioxide and oxygen was prepared, well mixed, and several samples were taken off. These were analyzed in duplicate in each of three machines by the short-cut method just described and in one machine by the standard Haldane procedure. The results are shown in Table II.

TABLE II

TYPE OF ANALYTICAL PROCEDURE		ANALYSIS NO. 1	ANALYSIS NO. 2	MEAN	MACHINE
Standard Haldane Procedure	Carbon dioxide	3.67	3.65	3.66	A
	Oxygen	16.83+	16.84	16.84	
	Carbon dioxide + Oxygen	20.50+	20.49	20.50	
Short cut Procedure B	Carbon dioxide	3.67	3.68	3.67+	A
	Oxygen	16.83	16.84	16.83+	
	Carbon dioxide + Oxygen	20.50	20.52	20.51	
Short cut Procedure B	Carbon dioxide	3.66+	3.66	3.66	B
	Oxygen	16.83+	16.84	16.84	
	Carbon dioxide + Oxygen	20.49+	20.50	20.50	
Short cut Procedure B	Carbon dioxide	3.67	3.66	3.66+	C
	Oxygen	16.84	16.86	16.85	
	Carbon dioxide + Oxygen	20.51	20.52	20.51+	

SUMMARY

1. A method is described to obviate the flushing of the dead spaces in routine gas analysis.

2. In the case of a triple gas analysis, such as for carbon dioxide, acetylene, and oxygen, the number of visits to absorbing solutions is reduced to the order of half the usual number.

3. The error introduced thereby is only a small fraction of the average error of the gas analysis itself.

4. The method depends upon special points of construction in the gas analysis apparatus which are described in full.

Addendum: The determination of the volume of the residual dead space was estimated in the following way:

1. Pure oxygen was taken into the machine and used to flush out both pipettes *A* and *B* after which the menisci were raised to the *U* marks and the stopcocks turned to lock up practically pure oxygen in the residual dead spaces.

2. The oxygen in the burette was then replaced by air and by sufficient trips to pyro all of that gas removed so as to leave nitrogen only.

3. By appropriate turning of the stopcocks all 3 menisci were then set to the *L* marks and the burette volume was read.

4. By adequate reflushing of the system and trips to pyro all the oxygen now in the system was absorbed and the diminished burette volume read.

5. The difference of the two volumes gives the sum of the residual dead spaces. Since the dimensions of these are almost identical, each individual dead space is one-half the total so obtained.

I wish to express my thanks to Arthur H. Thomas Co., of Philadelphia, for their cooperation in making all the stopcocks and most of the experimental glassware required for the work. They now make the complete apparatus.

DETECTION OF BLOOD BY MEANS OF CHEMILUMINESCENCE*

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THE chemiluminescence of certain organic compounds has aroused considerable academic interest in recent years. The two most active substances are: 3-aminophthalhydrazide (luminol) and dimethyldiacrydil nitrate which, by oxidation, exhibit an intense luminescence that so far surpasses all such known phenomena.

Gleu and Pfannstiel,[1] and Wegler[2] have recently made a thorough investigation of the 3-aminophthalhydrazide and other substituted hydrazides, and described new methods for their preparation. The 3-aminophthalhydrazide was first synthetized by Schmitz,[3] under the direction of Curtius, from the ester of the hemimellitic acid. Later on Curtius and Semper[4] obtained it from 3-nitrophthalacid-1-ester by boiling with hydrazine hydrate. They noticed the very strong bluish fluorescence in acid solution, but the most interesting property, the chemiluminescence, escaped their observation.

The 3-aminophthalhydrazide exhibits an intense blue chemiluminescence, by oxidation in alkaline solution, which was observed for the first time by Lommel in the Research Laboratories of the I. G. Farbwerke in Leverkusen, Germany. Lommel did not publish his observation but interested Albrecht[5] in studying the phenomena from a physicochemical standpoint. Harvey[6] found

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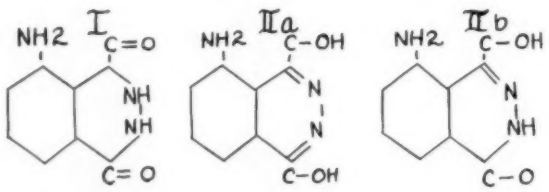
that chemiluminescence of the alkaline solution of 3-aminophthalhydrazide appears by anodic oxidation, or through activated molecular oxygen (activated by metals). Harvey considered the appearance of luminescence a criterion of active oxygen.

In 1935 Hundress, Stanley, and Parker[7] published a method for the preparation of 3-aminophthalhydrazide, and named the substance luminol. Harris and Parker[8] made physicochemical measurements of the quantum output of the chemiluminescence produced by luminol. According to Gleu and Pfannstiel, a convenient method for the preparation of pure 3-aminophthalhydrazide consists of boiling benzisoxazolon -4-carbonic acid with a strong aqueous solution of hydrazine. After diluting with water and cooling, yellow crystals of pure hydrazide are obtained. If the benzisoxazolon -4-carbonic acid is boiled for a longer time with hydrazide, or the yellow hydrazide treated with boiling hydrochloric acid, pure white hydrazide is obtained in good yields. The light yellow 3-aminophthalhydrazide is a phthalazine derivative existing in three tautomer forms.

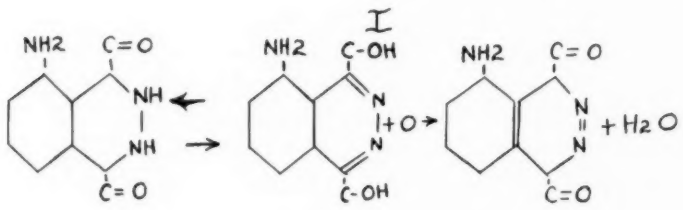


Amino-1-4 dioxo-phthalazindihydride | Amino-1-4 dioxi-phthalazine | Amino-1-oxi-4 oxo-phthalazindihydride

In dilute acids it is only slightly soluble. Such solutions probably contain form I and give a strong blue fluorescence. The addition of oxidizing agents like hypochlorites, does not produce luminescence. Forms IIa and IIb exist in alkaline solution. The 3-aminophthalhydrazide is easily soluble in alkali and forms well-defined monoalkali salts. The solution exhibits a very weak green fluorescence but gives an intense blue luminescence by oxidation with sodium hypochlorite. The aqueous solution of 3-aminophthalhydrazide gives a strong blue fluorescence, as do also the solid substances; forms I and II are probably combined into a salt. Chemiluminescence is only produced by oxidation in alkaline solution of form II. Originally calcium hypochlorite was used as an oxidizing substance, but other oxidizing substances like potassium ferricyanide, ammoniacalic copper solution, manganese peroxide, colloidal platinum, blood and vegetable peroxidases produce the same effect. Peroxide oxidizes slowly and produces a weak but continuous luminescence which increases with the elevation of temperature. Luminescence may continue for months at room temperature, but is weak in intensity. Persulfates have a similar action.

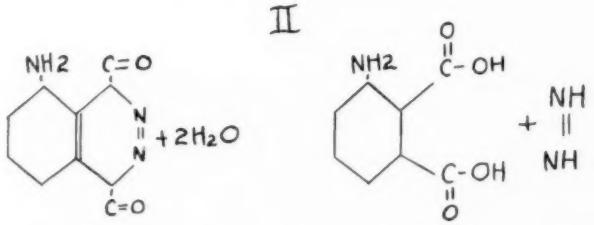
The 3-aminophthalhydrazide is rapidly oxidized with the formation of aliphatic acids and becomes brown in color. If the amino group is acetylated, acetyl 3-aminophthalhydrazide is formed and does not give a brown discoloration by oxidation; the luminescence is slightly green but very intense. The first phase of the reaction is the oxidation of the hydrazide to an azo compound.

I



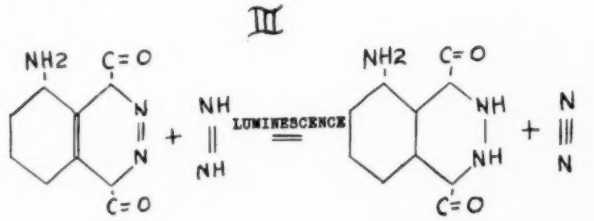
The azo compound has not been isolated so far. During its formation energy is stored. The azodiazyles are not water stable and hydrolyze almost completely in the presence of alkali.

II



The hypothetic intermediary product, the dimid, reacts with another molecule of the azo compound.

III



The last two equations demonstrate the luminescent process. The oxidation, therefore, is not the direct cause of the luminescence, but only serves to produce the azo compound. If this compound is formed, luminescence only appears after the addition of alkali. The oxidation of 3-aminophthalhydrazide forms an azo compound which is again transformed into an activated fluorescent aminophthalhydrazide.

Albrecht had already noted that blood, as well as fresh potato juice, produced a strong luminescence in the presence of hydrogen peroxide. Gleu and Pfannstiel confirmed Albrecht's observation. Boiled vegetable peroxidases did not give a luminescence. Pure hematin produced the most brilliant luminescence.

Upon the suggestion of Gleu and Pfannstiel, Specht[9] called attention to the forensic detection of blood stains by means of chemiluminescence. Specht's extensive investigations with fresh and old blood stains, as well as other stains which simulate blood, like colored preserves, milk, coffee stains, organic and inorganic dyestuffs (eosin, scarlet red, etc.), sperma, saliva, urine, feces, and other blood fluids, did not give a luminescence. Wallpaper, fabrics, leather, moldy bread, fungous cultures, oils and oil colors, varnish, mineral

oils, wax, shoe cream, soil, clay, sand, various stones, wood, grass, leaves, metals (copper, iron, brass, lead and zinc), did not produce a luminescence. Other metal oxides, especially rust, which in practice is often contaminated with blood and reacts strongly with hydrogen peroxide, do not react with luminol. Specht demonstrated the practicability of the method by spraying bushes, stone walls, rusty fences, etc., with blood; after exposure to sunlight and rain, luminol made the blood stains distinctly visible so that they could be photographed in the dark. Blood could also be detected in water, soap water, and sewage. A weak luminescence may be produced in the presence of hypochlorites, but with the careful addition of sodium hydroxide a momentary marked lighting will appear. Blood stains on hands could also be detected easily.

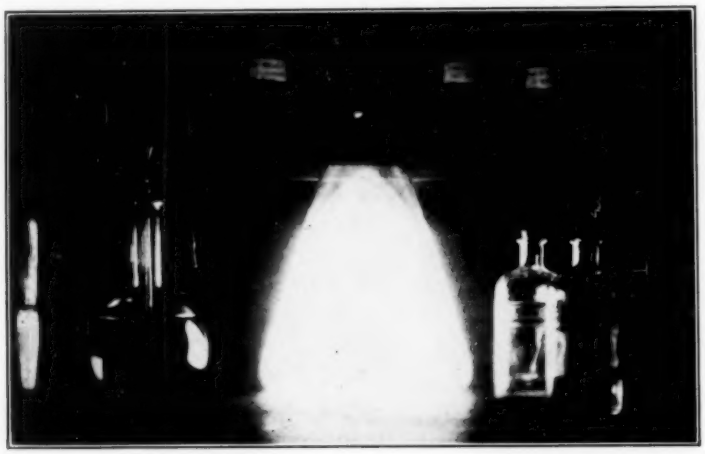


Fig. 1.—Model experiment with hematin. Photographed with orthochromatic film, sensitized with ammonia. Exposure time: forty seconds.

The 3-aminophthalhydrazide which we used in our experiments was obtained from the Eastman Kodak Co. It is a yellow, amorphous substance, easily soluble in a sodium carbonate solution. It is probably a mixture of yellow and white hydrazide and exhibits a greenish-yellow fluorescence in ultraviolet light. This substance is difficult to dissolve in boiling hydrochloric acid, and the acid solution does not fluoresce. The alkaline solution exhibits a brilliant luminescence with sodium hypochlorite, potassium ferricyanide, ammoniacalic copper solution, manganese peroxide, hematin, and vegetable peroxidases.

Specht used a 0.1 per cent solution of luminol in 5 per cent sodium carbonate with the addition of 15 per cent superoxydol (Merck) or 0.1 per cent luminol in 100 c.c. of a 0.5 per cent solution of sodium peroxide, in his experiments. The concentration of the sodium carbonate can be reduced to 0.5 per cent without impairing the brilliance of the luminescence. The expensive superoxydol can be replaced by the cheaper 3 per cent commercial hydrogen peroxide by adding 10 or 15 per cent to an alkaline luminol solution. The brilliance of the luminescence diminishes in a short time (ten to fifteen min-

utes) with a soda alkaline solution of luminol, but when luminol is dissolved in a filtered saturated solution of calcium hydroxide, the luminescence is prolonged.

We can confirm Specht's findings as to the negative luminescence of the various above mentioned substances. While the test is primarily a presumptive one for blood, it greatly facilitates the detection of blood stains as large areas of suspected material can be examined rapidly. We detected blood stains on paper, fabrics, and iron pipes which had been exposed to sunlight and air for three years. Three-year-old putrefied blood also gave brilliant luminescence. Dried and decomposed blood gives a stronger and more lasting reaction than fresh blood. If the luminescence disappears, it may be reproduced by the application of a fresh luminol-hydrogen peroxide solution. Dried blood stains may be made luminescent many times. Fresh blood stains which have been allowed to dry for a short time, may be made more luminescent if they are first sprayed with a 1 or 2 per cent solution of hydrochloric acid, which hydrolyzes the hemoglobin and produces acid hematin. Application of the alkaline luminol solution will give a strong luminescence.

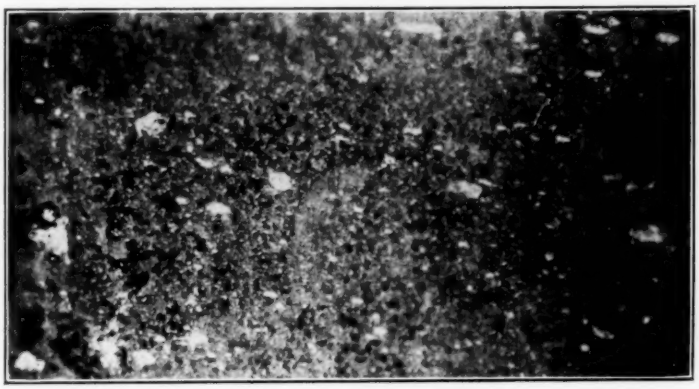


Fig. 2.—Rusty steel plate with oil paint and varnish spots contaminated with blood stains ten days old. Photographed by daylight.

Carbonized blood can also be detected if extracted with boiling dilute hydrochloric acid (10 per cent), the filtrate made alkaline and mixed with luminol and hydrogen peroxide. The remaining carbon, if still containing a sufficient amount of hematin, will also react with luminol if alkalinized. The chemical reaction that takes place between luminol, hematin, and hydrogen peroxide, is as follows: The nascent oxygen of the hydrogen peroxide is transferred to the bivalent iron of hematin which oxidizes the luminol and produces the luminescence.

Luminol reacts with animal, as well as with human, blood and is, therefore, only a general reagent for peroxidases. The advantage of luminol for the detection of blood, is its extreme sensitiveness; hematin can be detected in a dilution of 1:100,000,000. If blood stains are detected with luminol, they must be differentiated further as to their specific nature. Luminol does not interfere with spectroscopic, chemical, or precipitation tests, for the definite

identification of blood. Hematin and hemochromogen crystals of dried blood were obtained after repeatedly treating blood with luminol. The precipitin test can be applied if the blood is not decomposed.

Reagents.—Dissolve 0.1 gm. of luminol in 100 c.c. of a 5 per cent solution of chemically pure sodium carbonate. This solution may be kept indefinitely. Add 15 to 20 c.c. of a 15 per cent superoxydol solution (Merck) or the same amount of a 3 per cent commercial hydrogen peroxide to 100 c.c. of the luminol solution. If sodium peroxide is used in place of hydrogen peroxide, add 1 gm. to 100 c.c. of the luminol solution. Solutions should be made with distilled water and stored in clean bottles. The hydrogen peroxide or sodium peroxide should be added just before making the test. Traces of metal salts or free halogens will produce a slight luminescence, but this does not interfere with the test.

Fig. 3.—Same as Fig. 2 sprayed with luminol peroxide and photographed in darkroom. Orthochromatic film. Exposure time: two minutes.

Procedure.—To detect fresh blood in solution, it is preferable to transform hemoglobin into hematin. The solution is acidified with a few drops of chemically pure concentrated hydrochloric acid, boiled for a few minutes, cooled, and alkalized with a chemically pure sodium carbonate solution. Boiling will destroy vegetable peroxidases which also react to luminol. Add 5 to 10 c.c. of the luminol-peroxide reagent to the alkalized solution. If blood is present, a brilliant luminescence will immediately appear. The duration of the luminescence depends upon the amount of blood present. If the luminescence fades, it may be reproduced by adding a few cubic centimeters of hydrogen peroxide. This test is best performed in a darkroom.

To detect blood on paper, fabric, leaves, etc., it is more convenient to spray such areas with a 1 to 2 per cent solution of hydrochloric acid from a glass atomizer, to be followed in ten or fifteen minutes by spraying first with the sodium carbonate solution and then with the luminol-peroxide reagent. When blood is present, the smallest droplets become luminescent in the dark. If the luminescence fades, it may be reproduced by allowing the objects to dry and again spraying with the reagent.

To detect blood in carbonized material, it should be finely ground and extracted with a 10 to 15 per cent solution of boiling hydrochloric acid. After filtration of the undissolved residue, the extraction may be repeated several times, the combined filtrates evaporated to a small volume, and the solution alkalized and treated as previously described. The unsoluble residue may be suspended in a 5 per cent sodium carbonate solution and mixed with the reagent. If the hematin is not completely extracted, the undissolved particles will produce a distinct luminescence.

Conclusion.—The 3-aminophthalhydrazide (luminol) in soda or earth alkaline solution, in the presence of small amounts of hydrogen peroxide, is a general reagent for peroxidases like leucomalachite green, benzidine, phenolphthalein, etc. While the leucoaniline bases give a distinct color reaction in combination with hydrogen peroxide, luminol exhibits a brilliant luminescence in the dark. The luminescence can be photographed with orthochromatic plates or films, with an exposure time of from five to sixty seconds. Luminol is an extremely sensitive reagent for hematin. Inorganic catalyzers, such as hypochlorites, manganese peroxide, ferricyanide, colloidal platinum, osmium tetroxide, and gold chloride do not come into consideration in forensic cases. However, they may all be easily identified by chemical reactions. Other materials and substances in common daily use do not give luminescence.

REFERENCES

1. Gleu, Karl, and Pfannstiel, Karl: Ueber 3-Aminophthalsäure-hydrazid, J. prakt. Chem., N. F. **146**: 137, 1936.
2. Wegler, Richard: Chemilumineszenz cyclischer Hydrazide, J. prakt. Chem., N. F. **148**: 136, 1937.
3. Schmitz, A. J.: Diss. Heidelberg, 1902.
4. Curtius, T., and Semper, A.: Verhalten des 1-Aethylesters der 3-Nitrobenzol-1-2 carbonsäure gegen Hydrazin, Ber. **46**: 1162, 1913.
5. Albrecht, H. O.: Über die Chemilumineszenz des Aminophthalsäure-hydrazids, Ztschr. f. physiol. Chem. **136**: 321, 1928.
6. Harvey, N.: Luminescence During Electrolysis, J. Physiol. Chem. **332**: 1456, 1929.
7. Hundress, E. H., Stanley, L. N., and Parker, A. S.: The Preparation of 3-Aminophthalhydrazide for Use in Demonstration of Chemiluminescence, J. Am. Chem. Soc. **56**: 241, 1934.
8. Harris, L., and Parker, A. S.: The Chemiluminescence of 3-Amino-phthalhydrazide, J. Am. Chem. Soc. **57**: 1939, 1935.
9. Specht, W.: Die Chemilumineszenz des Hämins, ein Hilfsmittel zur Auffindung und Erkennung forensisch wichtiger Blutspuren, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**: 225, 1937.

A MODIFICATION OF THE BROWN ANAEROBE JAR*

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TWO anaerobe jars, both based upon the Laidlaw[1] principle, appeared in the literature simultaneously in 1921. Brown[2, 3] described a platinized or palladized catalyst system which was activated by an electrically heated resistance wire. Fildes and McIntosh[4] in England described a jar which was very similar and was activated by means of a resistance wire wrapped inside the catalyzing capsule. The British jar was made commercially available and became widely used. It was necessary for those interested in the Brown jar to make their own, since it was not placed on the market until recently.

To the casual reader these two jars would seem exactly alike in principle, but if one studies the two jars important differences will be observed. Some of these differences are basic in design. The resistance wire in the catalyst capsule of the Fildes and McIntosh jar is wound directly on some insulating material and the platinized asbestos packed loosely around the resistance coil and in contact with the wire gauze container. In the Brown jar the catalyst is bound to the insulator by the resistance wire which, therefore, heats the exposed surface of the platinized asbestos. An important difference is that in the Brown jar the catalyst is not allowed to touch the heat-dissipating wire gauze and, as Brown states, the safety principle of the Davy miner's lamp is utilized to minimize the danger of explosion.

Another basic difference is the method by which hydrogen is introduced into the jars. The Fildes and McIntosh jar has two gas connections by means of which hydrogen is passed through the jar before the electric current is turned on; the heated catalyst later serving to get rid of the remaining oxygen. The Brown jar has only one gas connection and, although it is possible to evacuate the jar before admitting hydrogen, this is not necessary. Thorough mixing of the hydrogen and air is accomplished by leading the hydrogen to the bottom of the jar by means of a rubber tube attached to the gas inlet. If the jar is not evacuated the collection of moisture on the sides of the jar serves to indicate that combustion has taken place.

The Brown jar as originally described was made from a converted museum jar; however, the lid to the present commercial model is made of bronze and the workmanship has been greatly improved. The glass stopcock has been replaced by a rubber tube and screw clamp, which Brown has found more satisfactory than either the stopcock or needle valves that require attention when they begin to wear. The rubber tube may be replaced when necessary.

Although the Brown jar in its present construction is quite safe, there may be some objection to a wire, bearing an electric current, passing through

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a mixture of hydrogen and air, since a spark from a broken connection might cause an explosion. To eliminate this possibility, I have developed a modification of the apparatus in which the electric wires do not pass into the jar and the resistance wire is isolated from the interior of the jar. The advantages of the Brown jar have been retained.

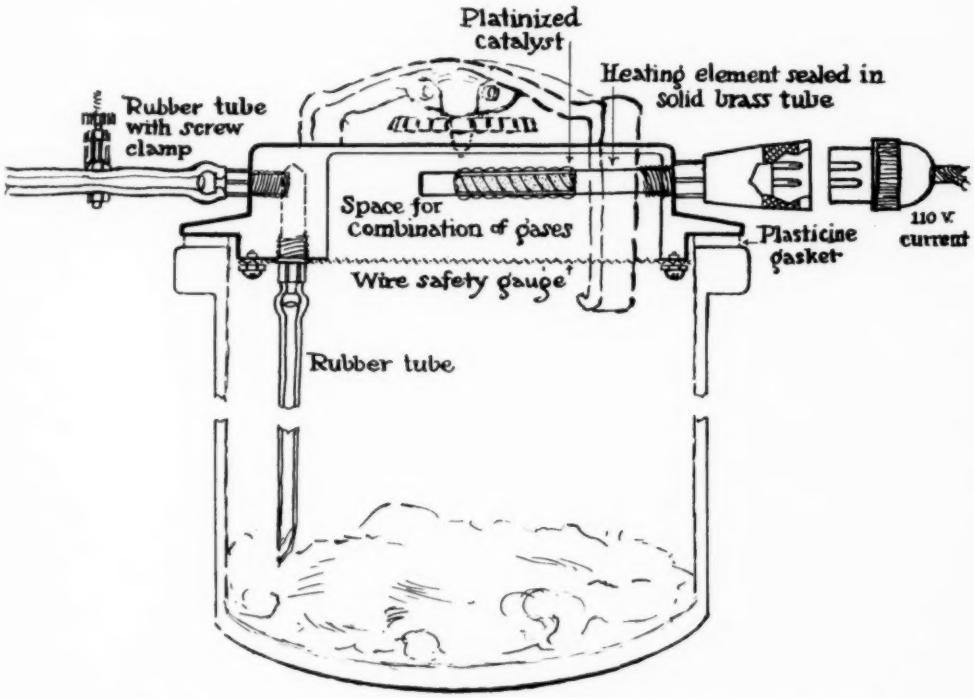

Fig. 1.—Diagrammatic illustration of cross section of jar showing construction.

The present modification of the lid (Fig. 1) has been so designed that it may lie flat on the table without danger of damaging electrical or gas connections. The catalyst capsule does not hang down, occupying space within the jar. The heating element, sealed within a brass tube, draws only 7 watts current and requires no rheostat or lamp bank resistance. Connection from the jar to direct or alternating 110 volt current is made by a conventional type fixture which plugs directly into the lid. No other wire connections are necessary.

When hydrogen is employed, the jar is used exactly as is the Brown jar except that no lamp bank or rheostat is required. The usual procedure is to attach the source of hydrogen to the jar until a pressure of 1 or 2 pounds is reached, then the electric current is turned on and the jar is left attached to both the hydrogen and electric current for thirty minutes. The collection of moisture within the jar is an indication that the jar is working satisfactorily. After being attached for one-half hour to the hydrogen, the screw clamp is closed and the electricity is discontinued. To start the jar and disconnect it requires only a few minutes.

This jar will operate very satisfactorily with illuminating gas, if the method described by Brewer and Brown[5] is followed. The use of this lid with illuminating gas simplifies the use of anaerobe jars, for it eliminates the hydrogen cylinder, reducing valve, generator, and lamp bank or rheostat, thereby reducing the cost of equipment to less than one-half.

SUMMARY

The advantages of the Brown anaerobe jar over jars of similar type have been pointed out and a modification has been described which possesses the following additional features: The electrical current does not pass through the jar, thereby eliminating the possibility of explosion due to sparking at loose or broken connections. No rheostat or lamp bank is required; the jar connects directly to the 110 volt current. The general design of the lid has been changed to conserve space and to aid in handling of the equipment.

REFERENCES

1. Laidlaw, P. P.: Some Simple Anaerobic Methods, Brit. M. J. **1**: 497, 1915.
2. Brown, J. Howard: An Improved Anaerobe Jar, J. Exper. Med. **33**: 677, 1921.
3. Brown, J. Howard: Modification of an Improved Anaerobe Jar, J. Exper. Med. **35**: 467, 1933.
4. Fildes, P., and McIntosh, J.: An Improved Form of McIntosh and Fildes' Anaerobic Jar, Brit. J. Exper. Path. **2**: 153, 1921.
5. Brewer, J. H., and Brown, J. Howard: A Method for Utilizing Illuminating Gas in the Brown, Fildes and McIntosh or Other Anaerobe Jars of the Laidlaw Principle, J. LAB. & CLIN. MED. **23**: 870, 1938.

AN OXYGENATOR WITH A LARGE SURFACE-VOLUME RATIO*

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THE most essential part of any apparatus for the perfusion of organs is the mechanism for oxygenating venous blood. In order to introduce an adequate amount of oxygen into a swiftly moving stream of blood, the blood must be spread out into a very thin film. Such a film has been produced in a variety of ways: by centrifugal force, by bubbles, by flow through a hanging cloth curtain, and by passage over glass beads. In general, the difficulties encountered are foaming, frothing, hemolysis from the trauma, and production of vasoconstrictor substances in the blood from mechanical agitation.

Most of the oxygenators which have been described contain a relatively large amount of blood in proportion to the rate of blood flow through the apparatus. To perfuse an entire animal[1] it was desirable to have a method of introducing oxygen which did not require a large volume of blood in the oxygenating apparatus. In other words, it was desirable that the ratio of the surface available for a film of blood to the volume of blood which the apparatus contained should be a large one. In addition to the large surface-

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volume ratio it was necessary for the oxygenator to accommodate varying rates of flow (100 to 500 c.c. per minute) without foaming. It was also essential to make the diffusing surface of the apparatus large enough to introduce sufficient oxygen to maintain life in a small animal.

A number of different types of oxygenators have been described. Hooker[2] in 1915 devised a revolving flat disk, onto the center of which a stream of blood was led from above. The revolving disk threw the blood in a thin film against the sides of an inverted glass bell jar. Hooker gave no figures as to the rate of blood flow, volume of blood contained, or amount of oxygen introduced per minute by this apparatus. The obvious drawbacks were the production of foam and the difficulty in ensuring the blood would remain in a film on the sides of the bell jar. Also in 1915 Richards and Drinker[3] described a method of oxygenating blood by allowing it to run through a silk curtain exposed to oxygen. As the blood saturated the curtain there were two surfaces exposed to oxygen. The rate of flow was 50 c.c. per minute and it introduced 3.75 c.c. of oxygen per minute. In 1922 Drinker, Drinker, and Lund[4] used a Hooker type of oxygenator which accommodated a blood flow of from 10 to 60 c.c. per minute. Bornstein[5] in 1926 oxygenated blood to perfuse the hind limb of a dog by passing blood through a glass chamber filled with glass beads. Oxygen was blown through this chamber in the direction of the blood flow. Blood flowed through the apparatus at a rate of 200 to 500 c.c. per minute. In 1928 Bayliss, Fee, and Ogden[6] devised an oxygenator consisting of a tier of rotating disks. The blood when thrown to the side of the apparatus from the top disk was again led into the center by a stationary funnel-shaped retaining wall which dropped the blood upon the second revolving disk. Blowing a stream of oxygen through the apparatus considerably increased its efficiency. With a tier of five disks the oxygenator held 54 c.c. and accommodated rates of flow up to 260 c.c. per minute. The surface area was approximately 1,500 sq. cm., and it was possible to introduce as much as 27 c.c. of oxygen per minute into the blood. Evans,[7] using this apparatus, experienced trouble with frothing. In 1932 von Euler and Heymans[8] devised an oxygenator consisting of a large spherical glass bulb into which blood and oxygen were injected under pressure through small tipped openings which were almost in contact with each other. The blood was thus blown in fine droplets against the inside of the spherical glass bulb. This apparatus could accommodate rates of flow up to 250 c.c. of blood per minute and introduced as much as 20 c.c. of oxygen per minute. The authors state that with "proper handling" no frothing occurred with defibrinated blood. Slight hemolysis was occasionally present. In 1933 Daly and Thorpe[9] described a modification of the Hooker type of oxygenator. It consisted of three horizontal revolving disks and three tall glass cylinders arranged concentrically. This apparatus had a surface area of approximately 11,000 sq. cm., held 300 c.c. of blood, and introduced about 17 c.c. of oxygen per minute. Cruikshank[10] in 1934 described an oxygenator consisting of a revolving spiral sheet of metal inside a horizontal cylinder. This apparatus had a surface area of approximately 7,500 sq. cm., contained 400 c.c. of blood, and was capable of introducing 34 c.c. of oxygen per minute.

It seemed to us that another and simpler way of producing a very even thin film of blood would be to direct the blood against the inner surface of a vertical revolving cylinder. The centrifugal force would ensure the maintenance of a thin even film. The blood would be collected at the bottom of the cylinder in a stationary cup, into which the revolving end of the cylinder would extend. The passage of the blood from the lower edge of the revolving cylinder to the walls of the stationary cup had to be accomplished without producing foam or hemolysis from trauma. With such an apparatus the blood would leave the lower edge of the revolving cylinder at a tangent to the cylinder, and when it struck the retaining wall of the stationary cup, its direction would be changed to that of a tangent to the stationary cup. It is obvious that if this change in the direction of the blood as it strikes the retaining sides of the stationary cup is kept minimal, less trauma and foaming will occur. Hence it was desirable that the space between the cup and the revolving cylinder should be as small as possible. It is also obvious that with any given gap between the cylinder and the cup, the change in direction of the blood will vary inversely with the diameter of the cylinder. It was with these principles in mind that the oxygenator here described was designed.

Apparatus.—The oxygenator consists of a vertical steel cylinder (Fig. 1*A*), 46 cm. in length, with an internal diameter of 14 cm. Roller bearings (*B*, *B'*) are tapped on both ends of the cylinder. The external surface of the lower end of the cylinder is planed off to a depth of 3 or 4 mm. so as to form a shoulder about 10 cm. above the lower end of the cylinder. In the upright position this shoulder rests upon the inner racer of the lower roller bearing (*B'*) and supports the weight of the cylinder (this detail is not indicated in Fig. 1). The cylinder is maintained in its upright position by three square upright pillars of steel (*C* is one of these pillars) firmly attached to a broad steel base (*D*). The outer racer of the lower roller bearing (*B'*) rests upon three blocks of steel (*E*), one projecting from the inner surface of each square upright column. The upper ends of the steel pillars are rigidly held by a flat piece of steel in the shape of a "Y" (*F*). Wing-tipped screws (*G*) pass through holes near the ends of the three arms of the "Y" into the upper end of each upright column. Two screws (*H*) pass through each upright column and impinge upon the outer racers of the upper and lower roller bearings. These six screws prevent rotation occurring in the outer racers and hold the cylinder firmly in position.

The greater part of the space within the revolving cylinder is occupied by a smaller stationary cylinder (*I*). This cylinder (*I*) is hollow and closed at both ends except for a metal tube (*J*) which extends through the cylinder from one end to the other. The upper portion of the tube (*J*) passes through a hole in the center of the heavy metal "Y" (*F*). This tube serves to convey oxygen to the bottom of the apparatus. The oxygen then diffuses upward in close contact with the film of blood and escapes at the top. The cylinder (*I*), by occupying most of the air space within the revolving cylinder (*A*), ensures a smooth upward current of oxygen over the film of blood on the inner surface of the revolving cylinder. The metal plate (*K*) closing the upper end of the cylinder (I) extends outward to overlie the upper end of the revolving cylinder

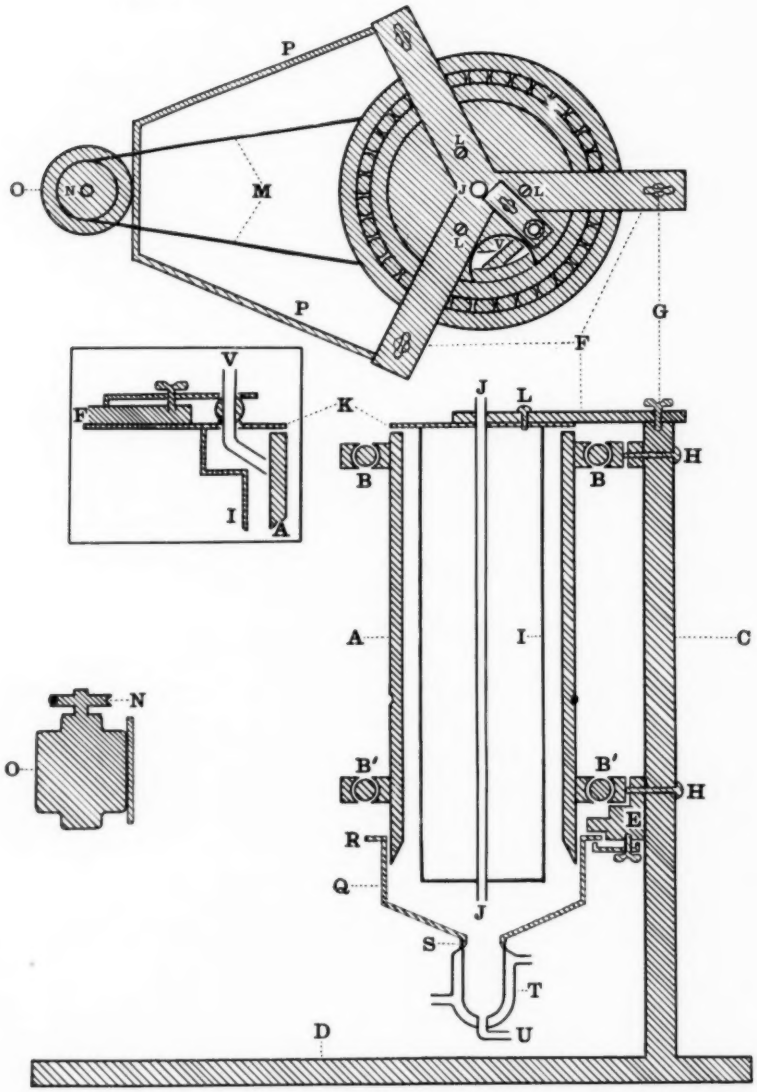

Fig. 1.—A vertical cross section and top view of the oxygenator. The drawing is not made to scale. The insert is a diagrammatic drawing of the tube which conveys the blood to the upper end of the inner surface of the revolving cylinder. For detailed description see text.

(*A*). The oxygen escapes at the top of the apparatus through the small gap between the metal plate (*K*) and the top of the cylinder (*A*). The central cylinder (*I*) is supported by three screws (*L*) clamping the plate (*K*) to the undersurface of the heavy metal "Y" (*F*).

Revolution of the large external cylinder (*A*) is accomplished by a pulley belt (*M*) passing around the cylinder in a groove on its outer surface. The belt then passes about a small pulley (*N*) fixed on the shaft of a one-sixth horsepower electric motor (*O*) which is mounted vertically. The electric motor (*O*) is supported by steel bars (*P*) projecting from two of the upright steel pillars.

The lower end of the vertical revolving cylinder is tapered from within outward to a sharp edge. Enclosing the lower end of the cylinder (*A*) is a stationary copper cup (*Q*) which receives the blood as it leaves the lower edge of the revolving cylinder. The upper margin of the cup has a small horizontal lip (*R*) extending outward. It is centered exactly in position by blocks (*E*) extending from the three upright columns which come into contact with the outer margin of this lip. The cup is supported in its position by clamping the lip upward against a projecting portion of the steel blocks (*E*).

The walls of the cup (*Q*) in its upper portion are at first vertical and then slope sharply inward to a central hole in the bottom, 3 cm. in diameter. From the undersurface of this hole there extends downward for about 2 cm. a small collar (*S*) with a one to ten taper. A double walled glass cup (*T*) has a glass collar with a similar taper at its upper end. This slips smoothly over the collar at the bottom of the stationary cup and is securely fixed there with deKhotinsky's cement. The glass cup receives the blood descending to it through the oxygenator, and the blood is led out from the bottom through a glass tube (*U*). The cup (*T*) is made of glass to permit observation of the level of the blood within it, and has a double wall through which warm water is circulated.

The blood enters the oxygenator at the top through a curved tube (*V*, insert Fig. 1) which pierces the top (*K*) of the stationary cylinder (*I*) and approaches the inner surface of the revolving cylinder (*A*) at an acute angle (top view, Fig. 1). The blood is thus directed against the upper part of the inner surface of the revolving cylinder with relatively little change in direction. This is an important consideration in the avoidance of splashing and foaming. The details of clamping the tube (*V*) so as to permit adjustment in different directions can be seen in the insert at the left in Fig. 1, and in the view from above at the top. In the latter view the semicircular window cut in the top (*K*) of the cylinder (*I*) can be seen. This window permits adjustment of the tube (*V*) under direct vision. The window is closed when blood is being oxygenated to ensure an even outward flow of oxygen as it escapes between the plate (*K*) and the upper end of the revolving cylinder.

Lamb's blood was used to test the efficiency of the apparatus. The blood was obtained from an abattoir and kept in an icebox when not in use. No observations were made with blood more than forty-eight hours old. There was always some hemolysis present in the blood obtained from the abattoir. The hemolysis was slightly more pronounced after prolonged passage, one hour or more, of the blood through the oxygenator.

The observations were made as follows: Pure nitrogen was blown through the gas intake tube of the oxygenator. The blood to be used was passed through the revolving oxygenator for from forty to sixty minutes, until it became definitely venous in color. It was collected under oil and a sample was withdrawn for determination of oxygen content and capacity. A two liter flask full of this blood, still protected from contact with air by a layer of oil upon its surface, was then passed through the oxygenator while a mixture of 95 per cent oxygen and 5 per cent carbon dioxide was blown through the gas inlet tube. Five per cent carbon dioxide was used because

it roughly approximated the carbon dioxide content of the alveolar air and would thus maintain the carbon dioxide content of the blood at an appropriate level during perfusion experiments.

The blood was led from the glass cup at the bottom of the oxygenator into a second flask and collected under oil. The passage through the oxygenator was started and stopped abruptly, and was timed with a stop watch. The total amount of blood passed through in this time was measured. A sample was then taken of the blood and the oxygen content and capacity were determined.

TABLE I

OBSERVATION NO.	BLOOD SAMPLE	OXYGEN CAPACITY	OXYGEN CONTENT	SATURATION	OXYGENATOR RATE	BLOOD FLOW	OXYGEN INTAKE	OXYGEN INTAKE PER C.C. BLOOD
		vol. %	*vol. %*	*%*	*r.p.m.*	*c.c./m.*	*c.c./m.*	*c.c./m.*
1	First	20.75	16.56	80.0	589	253	10.3	0.0407
	Second	21.00	20.65	98.0				
2	First	17.45	13.53	78.0	522	534	18.0	0.0337
	Second	17.20	16.90	98.0				
3	First	16.36	10.60	65.0	284	487	14.0	0.0285
	Second	16.05	13.46	84.0				
4	First	15.95	12.20	76.0	460	458	7.4	0.0160
	Second	16.05	13.82	86.0				
5	First	16.56	11.96	72.0	475	531	14.0	0.0264
	Second	16.18	14.60	90.0				
6	First	17.00	11.83	69.0	419	392	8.6	0.0219
	Second		14.02	83.0				
7	First	18.30	9.29	51.0	491	465	9.7	0.0209
	Second	17.37	11.38	65.0				
8	First	17.37	11.38	65.0	482	504	12.5	0.0248
	Second	17.50	13.87	79.0				
9	First	17.85	5.42	30.4	450	251	15.1	0.0601
	Second	18.15	11.45	63.0				
10	First	18.05	3.57	19.8	251	237	9.5	0.0401
	Second	17.85	7.60	42.6				
11	First	17.42	3.68	21.1	450	222	5.39	0.0243
	Second	17.50	6.11	34.9				

The efficiency of the oxygenator in introducing oxygen into venous blood can be seen in Table I. The rate of revolution of the cylinder, the blood flow, and the oxygen saturation of the venous blood varied in different observations. The oxygen intake per minute was from 5.39 to 18.0 c.c. The oxygen intake per cubic centimeter of blood per minute varied from 0.0160 to 0.0601 c.c. The surface area of that portion of the apparatus which is covered by a film of blood is 2300 sq. cm. Varying the flow of blood through the apparatus from 50 to 600 c.c. per minute does not result in foaming. A reservoir of 15 c.c. of blood was maintained in the glass cup at the bottom of the oxygenator in order to avoid the possibility of air being drawn into the artery of the animal to be perfused. Aside from this reservoir, 35 c.c. of blood are contained upon the filming surfaces during operation of the apparatus.

By rough calculations the oxygenator of Bayliss, Fee, and Ogden has a surface-volume ratio of 28; the oxygenator of Daly and Thorpe, a ratio of 37; and the oxygenator of Cruikshank, a ratio of 30. The oxygenator here described has a surface-volume ratio of 66.

SUMMARY

An oxygenator with a large surface-volume ratio has been described. It is capable of introducing as much as 18 c.c. of oxygen per minute and accommodates rates of flow of from 50 to 600 c.c. per minute without foaming. It has been used satisfactorily in a large number of perfusion experiments for a period of more than two years, and is thought to be a simple and convenient method of introducing oxygen into blood for perfusion purposes.

REFERENCES

1. Gibbon, J. H., Jr.: Artificial Maintenance of Circulation During Experimental Occlusion of Pulmonary Artery, Arch. Surg. **34**: 1105, 1937.
2. Hooker, D. R.: The Perfusion of the Mammalian Medulla: The Effect of Calcium and of Potassium on the Respiratory and Cardiac Centers, Am. J. Physiol. **38**: 200, 1915.
3. Richards, A. N., and Drinker, C. K.: An Apparatus for the Perfusion of Isolated Organs, J. Pharmacol. & Exper. Therap. **7**: 467, 1915.
4. Drinker, C. K., Drinker, K. R., and Lund, C. C.: The Circulation in the Mammalian Bone Marrow, Am J. Physiol. **62**: 1, 1922.
5. Bornstein, A.: Über Durchblutungsversuche an der überlebenden Hundeextremität, Arch f. exper. Path. u. Pharmakol. **115**: 367, 1926.
6. Bayliss, L. E., Fee, A. R., and Ogden, E.: A Method of Oxygenating Blood, J. Physiol. **66**: 443, 1928.
7. Evans, C. L., Grande, F., and Hsu, F. Y.: Two Simple Circuits for Blood-Fed Hearts, Quart. J. Exper. Physiol. **24**: 283, 1934.
8. von Euler, U. S., and Heymans, C.: An Oxygenator for Perfusion Experiments, J. Physiol. **74**: (Proc.) 2, 1932.
9. Daly, I. de B., and Thorpe, W. V.: An Isolated Mammalian Heart Preparation Capable of Performing Work for Prolonged Periods, J. Physiol. **79**: 199, 1933.
10. Cruikshank, E. W. H.: A Magnetic Blood Oxygenator, J. Physiol. **82**: 26, 1934.

DETERMINATION OF SULFANILAMIDE IN TISSUE, URINE, AND BLOOD*

A Modification of Marshall's Method

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IN A STUDY of the distribution of sulfanilamide in the body two difficulties were met in using Marshall's original method[1-4] for the determination of this substance: (1) a brown interfering color developed, and (2) quantitative recovery of the drug added to tissue and fluid samples before extraction was not always possible.

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No criticism is offered here of Marshall's improved method for the determination of sulfanilamide.[5] However, the subjoined methods are capable of returning a high degree of accuracy, and are convenient in application and simple in technique.

In determining the effect of hydrochloric acid on sulfanilamide it was noted that in the solution neutralized with sodium hydroxide the brown color developed. Sulfanilamide solutions containing 0.25 to 1.5 per cent sodium chloride also developed this color when analyzed.

Standard solutions of sulfanilamide containing excesses of silver nitrate from 1 to 5 per cent were repeatdly compared to standard solutions of sulfanilamide in distilled water alone, and the results of these trials uniformly indicate that these excesses of silver nitrate have no detectable effect on the determination of the drug. Hence this substance is used to remove the chlorides.

Materials Used:

1. Alcohol, U.S.P., 95 per cent.
2. Aqueous solution of silver nitrate, 20 per cent weight per volume.
3. Aqueous solution of para-toluenesulfonic acid, 20 per cent weight per volume.
4. Freshly prepared 0.1 per cent aqueous solution of sodium nitrite.
5. Alcoholic solution of dimethylamino-alpha-naphthylamine, 0.4 per cent.
6. Appropriate standard solutions of sulfanilamide.
   (a) Aqueous solutions, 1 mg. per cent and 2 mg. per cent sulfanilamide.
   (b) Alcoholic solutions, 1 mg. per cent and 2 mg. per cent sulfanilamide.

Standard solutions can be prepared from stock solutions, 100 to 400 mg. per cent, which keep well if stoppered and kept from light. Fresh dilute standards should be prepared about every three days.

Extraction From and Determination in Tissue.—Twenty to 40 gm. samples are ground with clean dry sand, dried on a steam bath, and ground to a uniform powder, which is packed into a U.S.P. type percolator and washed thoroughly with petroleum benzene, the excess of which is removed by suction until the column of powder is dry. This procedure obviates the loss of material by removal from the percolator. The fat-free residue is macerated for twelve hours with 95 per cent alcohol, and then percolated, a total of 500 c.c. of percolate being collected.

To 5 c.c. of the well-mixed percolate, and simultaneously to 5 c.c. of an appropriate alcoholic standard, are added 5 c.c. of water and 1 c.c. of 20 per cent silver nitrate solution. After standing five minutes, 1 c.c. of 20 per cent para-toluenesulfonic acid solution is added, followed by 1 c.c. of freshly prepared 0.1 per cent solution of sodium nitrite. Five minutes later, 5 c.c. of the 1:250 solution of dimethylamino-alpha-naphthylamine in alcohol are added. This mixture is agitated, allowed to stand ten to fifteen minutes, and then filtered until clear, using talc if necessary. The sample and standard solutions so prepared are then compared in a colorimeter.

In order to check the accuracy of the method as outlined for tissue, samples of 20 to 40 gm. (moist) of tissue, taken from the brain, heart, liver, spleen, kidneys, lungs, stomach, skin, and muscle, respectively, were ground up as directed with sand, and the mixtures placed in evaporating dishes on

the steam bath. To these moist tissue specimens before drying and extraction, a known volume of standard sulfanilamide solution in alcohol was then added. Upon such prepared, known samples, the process of extraction and determination was then applied as given above, with results as indicated in Table I.

TABLE I

DOG	SULFANILAMIDE ADDED	SULFANILAMIDE RECOVERED WITH $AgNO_3$	PER CENT RECOVERED
Brain, 20 gm.	10 mg.	10.0 mg.	100.0
Spleen, 25 gm.	10 mg.	10.0 mg.	100.0
Liver, 40 gm.	10 mg.	10.06 mg.	100.6
Heart, 35 gm.	10 mg.	9.8 mg.	98.0
Kidney, 30 gm.	10 mg.	10.12 mg.	101.2
Pancreas, 25 gm.	10 mg.	9.8 mg.	98.0
Muscle, 50 gm.	10 mg.	9.9 mg.	99.0
Skin and hair, 20 gm.	10 mg.	10.2 mg.	102.0

In testing the accuracy of the methods for tissue analysis, the known quantities of sulfanilamide were added to the moist, ground tissue samples before extraction, and not to the extractive liquid prepared from such samples, as was done in previous work.[4] By adding the drug to the moist tissue, the samples, we believe, more nearly resembled unknown tissues brought in for analysis, and offered a test of the efficiency both of the extractive and of the determinative processes.

Two mice injected intraperitoneally with 4 mg. and 3.6 mg. of sulfanilamide, respectively, were placed in beakers for one hour. They were then killed, ground, and prepared as a sample by the above method, adding to the sample the washings from the beakers used to house the animals. The process of extraction and determination, applied to these samples, gave the following results:

No. 1. Free sulfanilamide, 98 per cent; total sulfanilamide, 100 per cent.

No. 2. Free sulfanilamide, 96.7 per cent; total sulfanilamide, 103.4 per cent.

Unknown samples were prepared by one worker and analyzed by another with consistent recovery of ±3 per cent.

Determination of Sulfanilamide in Urine.—Urine is diluted 1:10 or 1:20 for comparison with a 1 mg. standard.

To 10 c.c. of such dilution, and simultaneously to 10 c.c. of the appropriate aqueous standard, is added 1 c.c. of 20 per cent silver nitrate solution. After standing five minutes, 1 c.c. of para-toluenesulfonic acid solution, 1 c.c. of sodium nitrite solution, and 5 c.c. of 1:250 alcoholic solution of dimethylamino-alpha-naphthylamine are added to each solution. Ten to fifteen minutes later the mixtures are filtered until perfectly clear, using talc if necessary, and the sample and the standard solutions so prepared are then compared in a colorimeter. The results here obtained represent the amount of the drug in the free state.

We have been able to verify the statement that in man and in various lower animals, excepting the dog, sulfanilamide is excreted in a free and a combined form. To determine the total amount excreted, 10 c.c. of diluted

urine and 10 c.c. of aqueous standard are treated separately with 1 c.c. of 20 per cent para-toluenesulfonic acid in a boiling water bath for ninety minutes. Twenty-five cubic centimeter test tubes, graduated at 10 c.c., were found to be convenient.

The solutions are then cooled, the volume made up to 10 c.c. with distilled water and then treated as for determination of free sulfanilamide in the urine.

The methods for urine were checked as completely as possible by adding known quantities of sulfanilamide to the urine, and proceeding as above. Results within ±3 per cent theoretical content were consistently reported. Color matches were satisfactory if silver nitrate was used; the omission of the silver nitrate resulted in the production of brownish colors in the samples.

Determination of Sulfanilamide in Blood.—One volume of fresh or oxalated blood is laked by the addition of seven volumes of water, about ten to fifteen minutes being allowed for the laking. Two volumes of para-toluenesulfonic acid solution are added, and the mixture is thoroughly shaken. After about five minutes, the mixture is poured upon a filter of a size just capable of holding the entire volume of the mixture. The filtrate is received in a volumetric flask or calibrated container marked at the total volume of the precipitation mixture, or ten times the volume of blood taken.

When the liquid has completely drained from the filter, the original flask is washed with a small volume of water, and this water poured over the precipitate on the filter. This is repeated, and the residue on the filter is thus carefully washed with small portions of water until the filtrate reaches the mark on the receiving flask.

To 10 c.c. of this acid filtrate, 2 c.c. of 20 per cent silver nitrate solution are added. After about five minutes, 1 c.c. of sodium nitrite solution and 5 c.c. of dimethylamino-alpha-naphthylamine solution are added (total volume here 18 c.c.). At the same time, to 10 c.c. of the appropriate aqueous standard, 1 c.c. of silver nitrate solution is added; after five minutes, 1 c.c. of para-toluenesulfonic acid solution, 1 c.c. of sodium nitrite solution, and 5 c.c. of the dimethylamino-alpha-naphthylamine solution are added (total volume 18 c.c.). After standing ten to fifteen minutes, the solutions are filtered until clear, using talc if necessary, and the clear solutions are then compared in a colorimeter. As was the case with the urine determination, this result expressed the blood content of free sulfanilamide. To determine the total drug contained in the blood sample, hydrolyze the 10 c.c. of the above acid filtrate. After such hydrolysis, results obtained by the determination method express the total sulfanilamide contained in the blood under examination.

In this method, as stated for blood, we have eliminated the use of acid standard solutions, because of the apparent instability of such acid standards upon standing—thus eliminating what may well be, in routine laboratory application, a source of appreciable error. Moreover, the accuracy attending the use of solutions acidified as above at the time of the determination has been uniformly established throughout the series of experiments upon blood samples.

The accuracy of the method given here as applied to blood was checked by taking blood samples from a series of dogs, laking them as directed with

water, but adding to the laking water known quantities of sulfanilamide. Precipitation, filtration, and determination were then carried out upon the samples so prepared, and results within 2 per cent of the amount added to the samples were uniformly recorded. Again in the case of blood it was repeatedly shown that the addition of silver nitrate solution was necessary for the removal of materials interfering with the formation of the color desired for the assay.

TABLE II

RESULTS ON A SERIES OF PREPARED BLOOD SAMPLES (DOG BLOOD)

SAMPLE	SULFANILAMIDE ADDED	SULFANILAMIDE RECOVERED WITH $AgNO_3$	PER CENT RECOVERED
Citrated blood, 10 c.c.	1 mg.	1.0 mg.	100.0
Oxalated blood, 10 c.c.	1 mg.	1.0 mg.	100.0
Oxalated blood, 10 c.c.	2 mg.	1.96 mg.	98.0
Fresh blood, 10 c.c.	1 mg.	0.997 mg.	99.7
Fresh blood, 10 c.c.	2 mg.	2.03 mg.	101.5

The method presented here was applied to samples obtained by adding known quantities of sulfanilamide to water used in laking blood, prior to precipitation and filtration, and hence indicates the efficiency of the process through the various stages.

Interfering Substances Other Than Chlorides.—In one case blood samples were taken from a dog after the dog had received several injections of adrenalin. These samples, after the addition of sulfanilamide and the application of the process given for blood, gave results nearly double the actual content of sulfanilamide. It was also known that aqueous solutions of adrenalin alone, when treated by the process given for the determination of sulfanilamide, gave a pronounced dark red color, capable of seriously clouding the accuracy of any determination of sulfanilamide color. Ephedrine was shown to have a similar reaction, and coupling colors were subsequently demonstrated with a number of amino compounds, notably guanidine, and many of the amino acids. These results indicate that sulfanilamide determinations made upon the blood samples of a patient may be influenced by other substances given the patient simultaneously with the sulfanilamide.

These substances are not removed by silver nitrate.

DISCUSSION

The fact that the addition of chlorides to a sulfanilamide solution intensifies the interfering color, while the use of silver nitrate removes it, indicates that the chlorides present in the material are responsible in large part for the difficulties encountered. Apparently the silver nitrate removes other substances as judged from the muddy brown precipitate.

In the determinations made upon blood samples, it was noted that 1 c.c. of silver nitrate solution was not sufficient to remove all the interference, although that quantity had been sufficient for the samples from tissue and urine. Increasing the quantity of silver nitrate to 2 c.c. effectively removed interferences.

CONCLUSIONS

1. A modification of Marshall's method for the determination of sulfanilamide in tissue, urine, and blood is given.

2. Silver nitrate is used to remove interfering substances, notably chloride. An excess does not interfere with the determination.

3. Consistent results within ±3 per cent are obtained.

4. Epinephrine, ephedrine, guanidine and other amino compounds interfere with this test.

We wish to thank the Department of Medical Research, Winthrop Chemical Co., Inc., for the sulfanilamide used in these experiments.

REFERENCES

1. Marshall, E. K.: Determination of Sulfanilamide in Blood and Urine, J. Biol. Chem. **122**: 263, 1937.
2. Marshall, E. K.: Determination of Sulfanilamide in Blood and Urine, Proc. Soc. Exper. Biol. & Med. **36**: 422, 1937.
3. Marshall, E. K., Cutting, W. C., and Emerson, K., Jr.: Acetylation of Para-aminobenzenesulfonamide in the Animal Organism, Science **85**: 202, 1937.
4. Marshall, E. K., Emerson, K., Jr., and Cutting, W. C.: The Distribution of Sulfanilamide in the Organism, J. Pharmacol. & Exper. Therap. **61**: 196, 1937.
5. Marshall, E. K., and Litchfield, J. T.: The Determination of Sulfanilamide, Science **88**: 2273, 1938.

THE OPTIMUM DOSE OF SULFANILAMIDE FOR GUINEA PIGS*

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ONTARIO, CANADA

WITH THE TECHNICAL ASSISTANCE OF AMELIA RUTH THOMAS, M.T.

THE optimum dosage of sulfanilamide necessary to combat infection in human beings effectively has been established fairly well. That amount which will maintain a free sulfanilamide level of 7 to 10 mg. per 100 c.c. of blood is considered requisite, except in genitourinary disease. To accomplish this, between 50 and 100 grains must be given daily, in divided doses. We, however, could find no information in the literature as to the amount of sulfanilamide required to maintain a similar blood level in guinea pigs. Investigators evidently have employed an empirical dosage in their experimental work. The following investigation was conducted, therefore, with a view to obtaining information on this question. It was felt that publication of the findings might be of value to others engaged in sulfanilamide studies.

EXPERIMENTAL

The animals used were guinea pigs, approximately 12 months old, weighing on an average 620 gm. A total of 150 pigs were used in preliminary tests.

The mode of administration of sulfanilamide was by stomach tube. A No. 7 French catheter was used for this purpose. A 10 per cent suspension of the drug was delivered into the tube by syringe through a No. 21 gauge needle.

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Suspensions of sulfanilamide sufficiently fine to pass through a No. 21 gauge needle can be made as follows: A 10 per cent hot solution is rapidly cooled to slightly below room temperature under a stream of cold water. As soon as crystals begin to form, the solution is shaken until it is cooled. In the event the suspension is not fine enough, the process is repeated, using more rapid cooling and more vigorous shaking.

Free sulfanilamide determinations were made on blood, using Marshall's method.[1] Owing to the difficulty of obtaining blood from guinea pigs, an animal was killed for each determination and bled from the jugular vein. In a few cases blood was obtained from the heart once, and sometimes twice, but thereafter the animals were killed for the third specimen. With very few exceptions 3 animals were tested for each period observed.

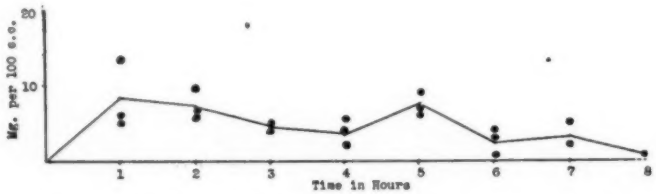

Fig. 1.—Blood sulfanilamide after a single 200 mg. dose.

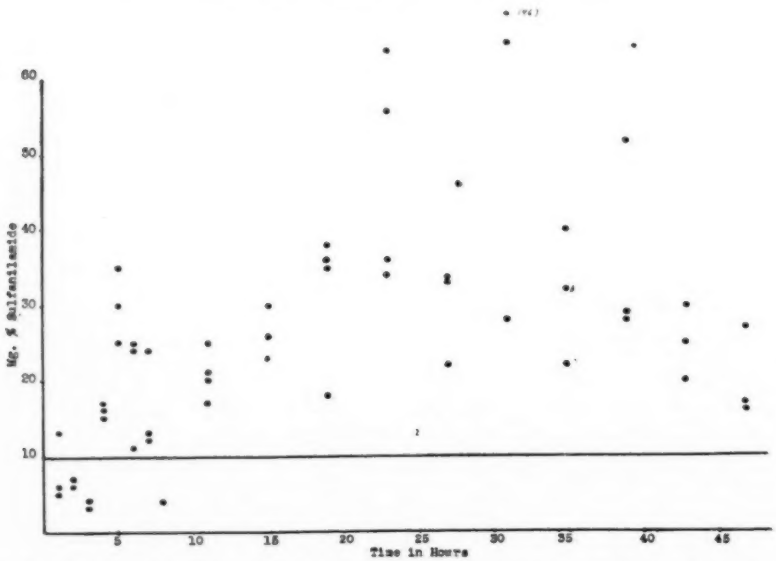

Fig. 2.—Blood sulfanilamide after repeated 200 mg. doses. Two hundred milligrams of the drug were administered at the following intervals: 0, 3, 7, 11, 15, 19, 27, 35, and 43 hours.

Fig. 1 shows the effect of a single 200 mg. dose. Throughout an eight-hour period, during which blood was analyzed each hour, all but one test revealed a value below 10 mg. per 100 c.c.

Fig. 2 illustrates the free blood sulfanilamide levels found when a 200 mg. dose was repeated after three hours, and again at four-hour intervals until nineteen hours had elapsed, following which 200 mg. were given at the end of every eight hours. Sulfanilamide determinations made every four hours

reveal that after the first eight hours the values were always greater than 10 mg. per 100 c.c. up to the end of the experiment. Very high values (over 50 mg. per 100 c.c.) were occasionally discovered.

Signs of toxicity were sometimes present at the twenty-third hour; ruffled coats, loss of appetite, slight cyanosis. These disappeared when the animals were put on the eight-hour dose regime.

COMMENT

It is felt that a sufficient number of pigs were used in the above experiments to permit the conclusion that 200 mg. doses, given at intervals as described, can be relied upon to elevate and maintain the free blood sulfanilamide to a level exceeding 10 mg. per 100 c.c. in all animals of the same approximate age and weight as those used in the foregoing experiments.

The sulfanilamide used in these experiments was obtained through Dr. Arnold Branch, of Ayerst, McKenna and Harrison, Ltd., Montreal.

The authors wish to express their gratitude to Dr. W. S. Stanbury for valuable advice and criticism.

REFERENCE

1. Marshall, E. K., Jr.: Determination of Sulfanilamide in blood and urine, J. Biol. Chem. 122: 263, 1937.

A MICROMETER CURVE MEASURING APPARATUS*

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THE problem of accurate measurement of recorded curves is a frequent one in a physiologic laboratory. The measurements most frequently required are time intervals, involving distance measurements in the horizontal, and wave amplitudes, involving distance measurements in the vertical direction. The accuracy of measurements depends in general on two things: (a) the sharpness of the onset of waves and peaks in the recorded curve and (b) the accuracy of the measuring apparatus. Aside from these factors, the range of variation in the physiologic response of a single feature must be considered when one curve, or one part of a curve, is to be compared with another. This, of course, is a factor which cannot be controlled instrumentally, but must be estimated by multiplying the number of separate observations.

A micrometer measuring device that was constructed in our shops and has proved satisfactory is pictured in Fig. 1. A heavy cast iron base *a*, 4¾ by 9⅛ by 1¼ inches; a second angular piece of cast iron *b*, 3 inches high and ½ inch thick, attached as shown to the base; and two steel bars, *c* and *d*, form the support for the movable carriage and microscope. One of the steel bars, *c*, is ½ inch square; the other, *d*, is somewhat larger and cut to a V-shape at the top. Both bars are 6¾ inches long and are firmly bolted to the upright support *b*. The carriage consists essentially of three parts as follows: (1) Two

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plates *e*, each 3⅝ by 1 5/16 by ¼ inches, cut out at each end to fit the steel bars, are attached to a metal plate below. The metal plate has an opening for the microscope. The anterior plate bears a micrometer screw *f;* the posterior plate, two spiral springs *s*, both of which press against the second part of the carriage *g* and serve to move this carriage in a forward and back direction between the limits of the two plates *e*. The anterior plate is provided with a clamp *h* for locking the under framework in any desired position along the steel bars. (2) A brass framework *g* provided with flanges *j* and fitting under the bars. This framework slides freely along the bars under the control of the micrometer head *f* within the limits of the plates *e*. (3) A brass plate *k*, which is fitted in slots in the upper part of the framework (2). This plate bears the microscope and slides laterally under the control of the micrometer head *m* and two spiral springs at the opposite end. The micrometer head and springs are attached to the framework (2).

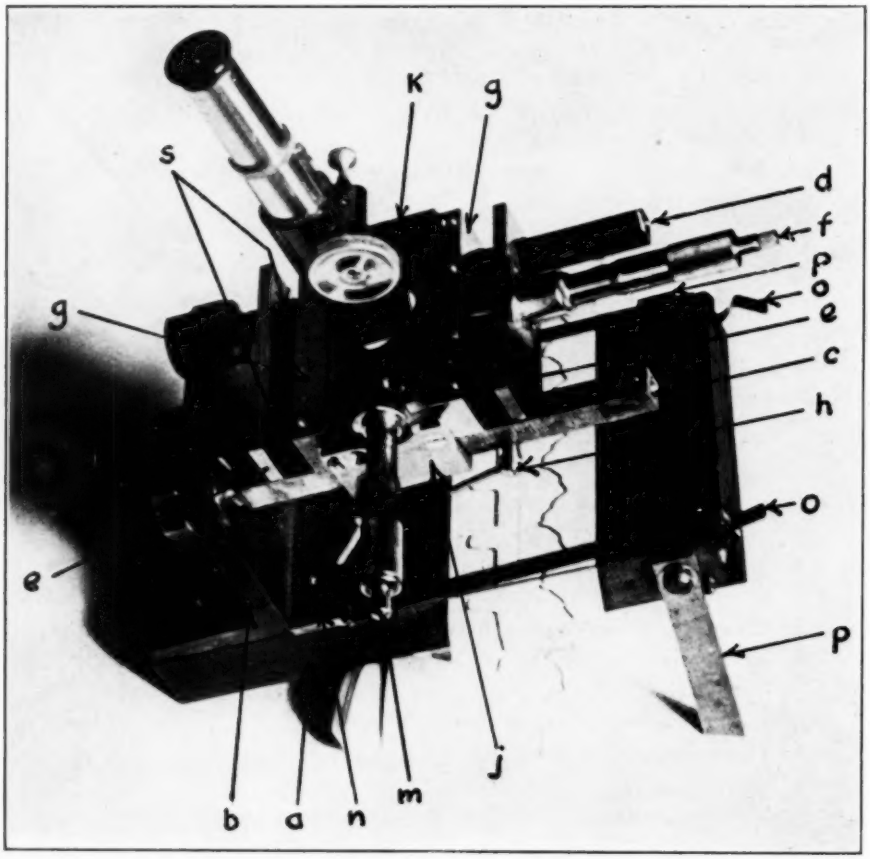

Fig. 1.

A brass plate *n*, the length of which is the same as the width of the base, and which is 1½ inches wide and 3/16 inch thick, is screwed to the base flush with the upright support *b*. This piece bears clips of spring brass *o*, the anterior ends of which clip into recesses cut in the base. The record to be measured is placed on the base with its edge in contact with the plate *n*, covered with a

glass plate and held firmly in place by the spring clips. Two collapsible metal legs *p* tilt the forward part of the base upward to place the microscope at a convenient angle.

The microscope has a magnification of 32 diameters and is provided with cross hairs and a focusing screw. It is model M101A of the Gaertner Scientific Co., Chicago, and was purchased at a cost of $26.00. The micrometer heads are of the usual type used in machine shops. They have a range of 25 mm. and read directly in 0.01 mm. divisions.

In using the apparatus the clamp *h* is loosened and the microscope carriage slid forward or backward to cover the portion of the curve to be measured. The clamp is now tightened and vertical measurements for curve amplitude made by the use of micrometer *f*, or horizontal measurements from which to obtain time intervals, made by the use of micrometer *m*. Light reflected from a small table lamp has been found to give sufficient illumination. When measuring curves recorded on transparent film, a piece of white paper or thin cardboard is placed back of the film. Illumination by transmitted light can be easily arranged for by cutting out a portion of the base and placing a mirror in a suitable position, but we have not found this to be necessary when tracings or photographic records show a fair degree of contrast.

As an indication of accuracy of measurement, the distance between two sharp peaks on two separate curves recorded simultaneously by cathode-ray oscillographs was measured fifty times, the cross hairs being moved away before each measurement. The record was made on film at a speed of 211 mm. per second. The average time interval between the two peaks in 50 measurements was 0.003465 second. The maximum difference between measurements was 0.000185 second (5.34 per cent of the mean). The standard deviation of the distribution is ± 0.0000548 second. The coefficient of variation is 1.58 per cent.

A METHOD OF ISOLATING LARVAE OF TRICHINELLA SPIRALIS FOR THE PREPARATION OF THE ANTIGEN USED FOR IMMUNOLOGIC REACTIONS IN TRICHINOSIS*

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ATTEMPTS to isolate the larvae of *Trichinella spiralis* free from infected muscles by using artificial gastric juice, as commonly employed in the preparation of the antigen for immunologic tests, frequently met with faliure. Careful scrutiny of the material thus obtained revealed a few microscopic fragments of undigested muscle fibers. It is highly probable also that a portion of the protein-cleavage products resulting from this chemical treatment may remain despite repeated washing of the material. It is generally considered that the presence of host proteins in such preparations may tend to give rise to nonspecific reactions in the immunologic tests for the diagnosis of trichinosis.

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The following method is, therefore, offered as a possible means of eliminating host proteins in the preparation of the antigen used in the tests.

The trichinella larvae are obtained from a rat or guinea pig, about two months old, previously infected with approximately 2,500 larvae isolated from infected muscles by the use of artificial gastric juice, as described by McCoy, Miller, and Friedlander.[1] On the sixteenth to the nineteenth day (the optimum being the seventeenth day) following infection, when the larvae had just threaded their way and come to lie along the long axis of the muscle fibers without definite coiling—the pre-encystment stage, the animal was killed. After the removal of skin, bone, and viscera, the carcass is ground up finely in a meat grinder. The ground material is then placed on two layers of gauze at the bottom of an ordinary potato ricer and enough lukewarm water is added to cover the material. The larvae are then readily forced out of the muscle fibers by gentle squeezing. The expressed juice, consisting chiefly of hemolyzed red blood cells and leucocytes together with many larvae, is collected in a flask. This is now filtered through a standard screen such as is made by the Tyler Company (200 meshes per inch, each opening measuring 0.074 mm.). The majority of viable larvae become coiled up by this treatment, and since the average diameter of these coiled larvae measures approximately 0.15 mm., they will not go through the mesh. The standard screen upon which the larvae are collected is then turned upside down, and the larvae collected thereon are washed down into a tall cylinder with sufficient amount of lukewarm water. This is left standing for a few hours until the larvae settle to the bottom of the cylinder. Baermann's isolation apparatus[2] of hookworm larvae may be also conveniently utilized for this purpose. After repeated washings with lukewarm water, the larvae are finally collected in a test tube and frozen by the use of the lyophile apparatus. The desiccated larvae are then pulverized and extracted with ether for forty-eight hours at room temperature, and then extracted with Coca solution. After centrifuging, the supernatant fluid is drawn off, filtered through a Seitz filter, and stored in ampoules for use as an antigen in immunologic reactions.

REFERENCES

1. McCoy, O. R., Miller, J. J., and Friedlander, R. D.: The Use of Intradermal Test in the Diagnosis of Trichinosis, J. Immunol. **24**: 1, 1933.
2. Baermann, G.: Eine einfache Methode zur Affindung von Ankylostomum (Nematoden) Larven in Erdproben, Med. Geneesk. Weltevreden, Feestbundel, pp. 41-47, 1917.

FREE CHLORINE AS A SOURCE OF ERROR IN BLOOD SUGAR DETERMINATIONS*

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RECENTLY we have experienced a difficulty in determining blood sugar by the Folin and Wu[1] method which we feel should be called to the attention of those working with this or similar methods. The difficulty was a rapid fading of the blue color upon dilution with water before making the colorimeter reading. Within five minutes the solution became colorless. Fading occurred even when a freshly prepared 1:4 dilution of acid molybdate solution was used as the diluting agent. After considerable search for the difficulty, it was finally found that the fading was due to the reoxidation of the blue compound to a colorless compound by free chlorine in the distilled water. The distilled water used was found to contain 0.2 part per million of free chlorine, as measured by the ortho-tolidine method.[2] Another source of distilled water was found to contain 0.1 part per million of free chlorine. This water did not cause complete fading, but fading was noticeable. When chlorine-free water was used, no fading was noted, the solutions retaining their blue color overnight.

It is felt that wherever chlorinated water is used in the preparation of distilled water, the danger of free chlorine distilling over is eminent. This is especially true where adequate control of the chlorination process is not maintained.

Although the effect of free chlorine has been noted only in connection with the Folin and Wu method for determining blood sugar, it is felt that it would interfere with other similar methods for sugar and also with any other method where color is the result of a reduction process.

REFERENCES

1. Folin, O., and Wu, H.: A System of Blood Analysis. Supplement I. A Simplified and Improved Method for Determination of Sugar, J. Biol. Chem. **41**: 367, 1920.
2. Standard Methods of Water Analysis, ed. 8, New York, 1936, American Public Health Association, pp. 19-25.

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A METHOD OF PANCREATECTOMY IN PIGEONS*

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KREBS† has used the breast muscle of depancreatized pigeons as a test object in the study of the influence of added insulin upon the intermediate products of carbohydrate metabolism. Great difficulty was encountered in these studies due to the high mortality attendant upon the removal of the duodenal loop containing the pancreas as well as the inability of maintaining these birds alive longer than three to four days.

Following Krebs' work, a study was made to find an adequate method of pancreatectomy in pigeons. The following is a brief description of the method finally adopted:

The pigeon is tied to a board by its feet and wings. It is given an ether anesthetic by means of dropping ether on a towel folded around its beak and head. The feathers are then plucked from its breast and abdomen. A curved incision, immediately below and parallel to the breast bone, is made through the muscle into the peritoneal cavity on the right side. The duodenal loop, which extends into the left side of the peritoneal cavity, can be recognized by its greater diameter, as compared to the rest of the bowel, and by the presence of the pancreas lying in the loop on both its ventral and dorsal aspects. The color of the pancreas varies from a rather bright red to a light tan. The distal portion of the duodenal loop is grasped in one hand with a square of surgical gauze soaked in saline. With a similar piece of gauze in the other hand, the portion of pancreas on the ventral surface of the loop is gently stripped from the mesentery on which it lies. It is necessary to exercise great care in securing the proper cleavage plane in order not to damage the blood supply to the adjacent bowel. Four of our series of 24 pigeons died as a result of such damage. In stripping off the pancreas it is necessary to maintain pressure on the bleeding points with the saline-soaked gauze. The large segment of pancreas on the ventral side strips quite readily, but the small portion, located at the proximal end of the loop, is removed only with difficulty. It is usually necessary to remove this piece of pancreas with a pair of fine forceps. When working in the proximal region of the loop, care has to be taken not to damage the bile duct which enters the descending limb of the duodenum near the small section of pancreas just mentioned. The portion of pancreas on the dorsal aspect of the loop is stripped off in the manner already described, care being taken as before not to damage the mesentery

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†Professor H. A. Krebs, of Sheffield University, was a guest worker in the Department of Medical Research in April, 1938.

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Fig. 1.

Fig. 2.

Fig. 1.—Pancreas in situ in duodenal loop, ventral aspect.
Fig. 2.—Pancreas in situ in duodenal loop, dorsal aspect.

Fig. 3.

Fig. 4.

Fig. 3.—Duodenal loop, eight days after pancreatectomy, ventral aspect. Note increased size of duodenum. Atonic condition probably result of interference of nerve supply.
Fig. 4.—Same as Fig. 3, dorsal aspect.

and to control any bleeding points with gentle pressure of the saline-soaked gauze. After pancreatectomy is complete, the loop is carefully replaced in the peritoneal cavity and closure is effected with white linen sutures.

Prior to pancreatectomy, blood glucose estimations were made, using 0.3 c.c. samples taken from the wing vein. The blood was precipitated by the method of Herbert and Bourne (1930) and true glucose determined with Harding and Downs' (1932) modified Shafer-Hartman reagent. The method of securing the blood is as follows: The feathers are plucked from the axillary region. Using a No. 23 needle, approximately 0.5 c.c. of blood is withdrawn from the wing vein. The blood is transferred to a glass slide, previously coated with paraffin, and then drawn from the slide by means of an 0.3 c.c. pipette. Even though many of the birds were starved for a period of twenty-four hours, it was difficult to secure a true fasting blood sugar on the pigeon because of the retention of food in the crop. Thus preoperative blood sugar levels varied from 124 to 200 mg. per cent. After operation, blood sugars were at first done daily, but it was felt that at least 4 birds died because of too frequent blood withdrawals. Hematomas may be prevented with care.

It is unnecessary to make blood sugar estimations more than once a week following pancreatectomy and even less frequently after the proper dose of insulin has been established. By the fourth day following pancreatectomy, the blood sugar might be expected to be between 200 and 350 mg. per cent. When the blood sugar was under 250 mg. per cent, 0.5 units of insulin (regular) was given daily, but when the values exceeded 250 mg. per cent, 1 unit was given. Without insulin a pigeon will live at least eight days following pancreatectomy. The extreme emaciation noted in depancreatized pigeons after the fourth day can be prevented by the addition of glucose and trypsin to the diet or by giving the birds glucose water to drink.

Out of 24 autopsies done on depancreatized pigeons, a small piece of pancreas was found in only 3 cases. Pigeons prepared and treated in the manner described herein have survived pancreatectomy for over thirty days. Figs. 1 and 2 show the pancreas in situ (ventral and dorsal views). Figs. 3 and 4 show the duodenal loop in a bird killed eight days after pancreatectomy (ventral and dorsal views).

SUMMARY

A procedure is described by which pigeons can be depancreatized and maintained for fairly long periods of time.

The morbidity and mortality following such procedure are extremely low.

The use of insulin in the depancreatized pigeon is described.

REFERENCES

1. Herbert, F. K., and Bourne, M. C.: The Non-sugar Reducing Substances of Human Blood, With Special Reference to Glutathione, Biochem. J. **24**: 299, 1930.
2. Harding, V. J., and Downs, C. E.: A Sensitive Copper Solution for Glucose Estimation, Canad. Chem. Met. **16**: 12, 1932.

AN ELECTRIC PULSE COUNTER*

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THE desirability for an automatic pulse counter has long been recognized. Although many attempts have been made to develop such equipment, no one has reported a counter which met all the requirements of a practical instrument. These requirements are stability, simplicity of operation, and accuracy.

Recent developments in amplification have made it possible to construct an apparatus that conforms to the requirements of a reliable pulse counter. By use of the instrument herein reported, it is practical to read the heart rate for any interval of time directly from the counter. In addition, the stability of the apparatus assures one of reliable data, even while the person is performing simple types of exercise.

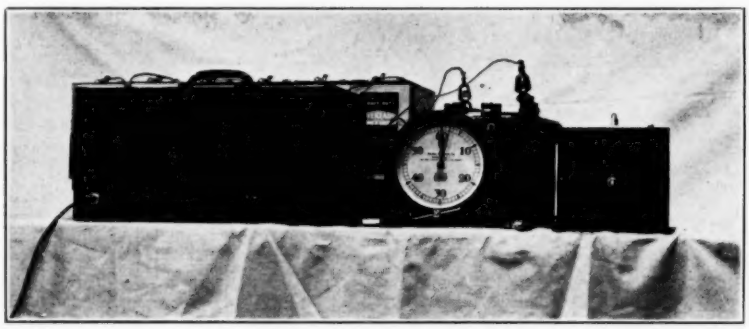

Fig. 1.—The pulse counter.

The principle of the pulse counter is the amplification of the action current produced by each contraction of the heart, sufficient to operate a relay which in turn operates an electromagnetic counter. The device is shown in Fig. 1.

The Electrodes.—The electrodes employed are direct-contact electrodes, the same as those used to connect a subject to the electrocardiograph. The position of the electrodes on the body is shown in Fig. 2. In order to insure good contact between the subject and the electrodes, a liberal amount of electrode jelly is employed. The electrodes are held in place by adjustable elastic bands, as shown in Fig. 2.

The input wires are crystal microphone cable, shielded and rubber jacketed. They are of sufficient length to insure free movement of the person during exercise.

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The electrodes offer no contact difficulties if properly connected to the person. If he is at rest, no precautions are necessary. However, if he is to perform exercise, it is well to free the contact surface from hair, fit the electrodes snugly with the elastic bands, and use an abundant amount of electrode jelly under and around the electrodes. Obviously, the lead in wires must make tight connection with the electrodes. Although soldering insures the best contact, the setscrews on the electrodes are adequate for tight connections.

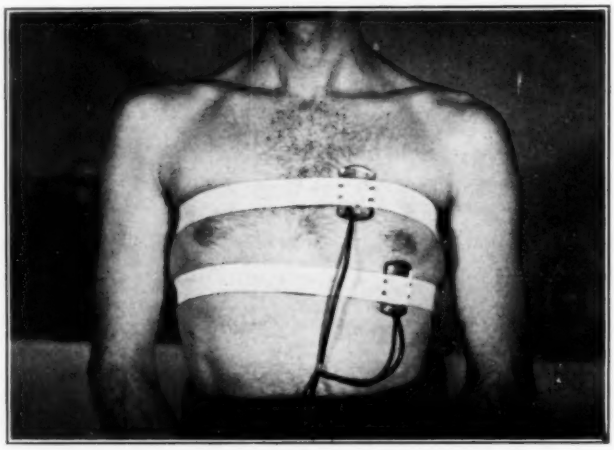

Fig. 2.—The electrodes.

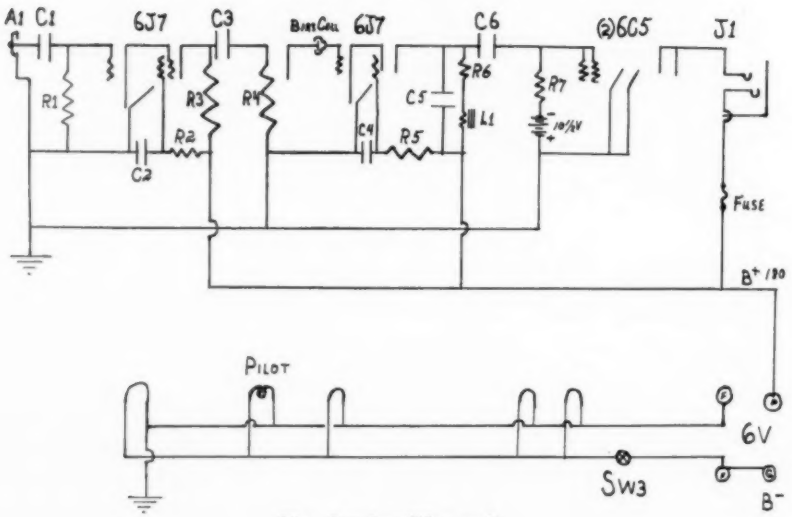

Fig. 3.—Amplifier unit.

R1 IRC BT1 20 meg.
R2 IRC BT1 2 meg.
R3 IRC BT1 0.5 meg.
R4 Yaxley "N" 0.5 meg.
R5 IRC BT1 0.5 meg.
R6 IRC BT1 9000 ohms.
R7 IRC BT1 0.5 meg.
C1 Aerovox 684 0.1 mfd.
C2 Aerovox 484 0.5 mfd.
C3 Aerovox 684 0.5 mfd.
C4 Aerovox 484 0.5 mfd.
C5 Aerovox 484 0.1 mfd.
C6 Aerovox 684 0.5 mfd.
L1 Thordarson T-93C20 250 Heneries.
Bias cell—Mallory 1 volt.
Fuse—Buss 8 AG ¼ amp.
A1 Amphenol PC1M connector.
J1 Yaxley No. 702B jack.
SW3 Yaxley No. 10 switch.
Pilot Yaxley No. 310R with No. 40 bulb.

The Amplifier.—The amplifier is a 3 stage high-gain impedance resistance coupled, designed to amplify the main peak of the electrocardiogram. The circuits employed are shown in Fig. 3. To prevent absorption of energy coming from persons with a high skin resistance, a high impedance input was employed. To this end, a 20 megohm input resistor was used, and the person further isolated from this high resistance by means of blocking condenser *C1*. The 20 megohm input resistor offers the further advantage that bias on the first tube is obtained by the drop caused by grid current flowing through the resistor. This circuit gives greater gain than can be obtained with other bias systems. The impedance *L1* in the plate circuit of the second tube is resonated at approximately 30 cycles by means of condenser *C5*. This tuned circuit has somewhat the effect of a low-pass filter.

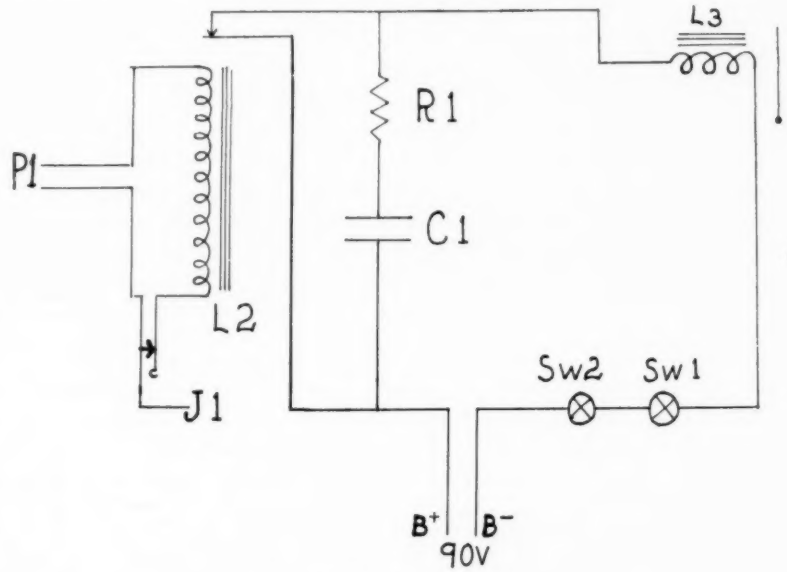

Fig. 4.—Relay counter unit.

P1 Yaxley No. 76 phone plug.
L2 Guardian No. 5—1000 G-1 relay.
L3 Western Electric 5AA message register.
R1 IRC BT ½ 500 ohms.
C1 Aerovox 684 0.05 mfd.
J1 Yaxley No. 701 jack.
SW1 Bud No. 743 push button switch.
SW2 Bud No. 1003 S.P.S.T. switch.

The output stage consists of two 6C5 tubes in parallel, biased to a "no-signal" plate current of about 0.5 Ma. Relay *L2* is activated by action currents passing through the amplifier, causing a change in the plate current as high as 5 to 6 Ma. Precautions have been taken to eliminate the possibility of a shock to the subject by using dissimilar connectors on the input and output circuits.

The relay is contained in the counter unit and is shown in Fig. 4.

The Counter.—The counter employed is a Western Electric message register, which is accurate up to a speed of 300 per minute. The counter unit contains an unusual feature in that the relay circuit contacts are normally closed, the counter being activated on the break rather than on the make, as is found in conventional arrangements. The arrangement of the counter is

shown in Fig. 4. This modification is necessary because of the short duration of the action current produced by the heart. It also eliminates interference caused by sparking of the relay contacts, permitting the apparatus to be operated in an unshielded room. Resistors *R1* and *C1* are connected across the relay contacts to further reduce any possible sparking. A normally closed push-button switch *(SW1)* has been included in the circuit to enable the counter to be set at a convenient number. A jack *(J1)* has been included in the relay coil circuit to enable a remote control switch or timer to be connected if it is desired.

Method of Operation.—The electrodes are fixed in place in the approximate line of the electrical axis of the heart, one over the apex and one over the base, which allows leading off the maximum voltage; the starting switches on the amplifier and relay system are closed, and the gain control on the amplifier is varied until each beat is recorded accurately on the counter. Too low amplification will result in missed beats, and too high amplification will register false beats on the counter. The relay counter unit should not be placed within 5 feet of an a-c circuit as the relay will flutter, giving false counts. Also the relay counter unit must be placed at least 1 foot from the amplifier as the vibrations of the counter may microphonically affect the tubes of the amplifier. Readings of the counter may be taken at desired intervals or the counter may be made to operate during any desired interval which is automatically started and interrupted by a timing device.

SUMMARY

An automatic pulse counter which is accurate, stable, and simple to operate, has been devised and described. It is capable of accurate counting even while a person is performing simple exercise. The pulse rate is read directly from the counter, thereby eliminating the objection of recording and then counting.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ERYTHROBLASTOSIS Foetalis, Macklin, M. T., Lamont, J. A., and Macklin, C. C. Am. J. Dis. Child. 57: 349, 1939.

A case of erythroblastosis foetalis accompanied by hemolysis and edema is reported in a newborn girl. There was nothing in the maternal history to explain the condition. Five of the seven pregnancies of the mother ended in miscarriage or premature delivery. It is suggested that the earlier products of conception may also have been examples of abnormal production of blood in which the defect was severe enough to interfere with further development.

It is also suggested that the defect may be one of actual derangement of blood formation in the fetus and not a persistence of a normal fetal blood picture.

ALLERGY in Childhood, Ratner, B. J. A. M. A. 111: 26, 1938.

No dogmatic conclusions can be drawn with respect to the onset and progress of allergic syndromes in childhood. This is due to the fact that our knowledge of the underlying mechanism of allergy is still far from complete.

It is apparent that allergy may start at an extremely early age. The newborn period is signally free, with the rare exception of an occasional instance of passive sensitization acquired in utero.

Eczema is the prevailing allergic syndrome in infants under 1 year of age, and foods are the prevailing reacting substance. Though food sensitivities were found in 100 per cent of our patients under 1 year of age, they are, nevertheless, not always the sole cause of the allergic syndrome. The allergic eczemas in many instances (59 per cent) are shown to be due largely to contact with environmental substances to which these same infants react.

Infants who react to egg and other food proteins which they have never ingested have most probably been actively sensitized in utero. The early allergic manifestations may be invoked by a passage of these substances through the breast milk in infants previously sensitized in utero. On the other hand, such potential sensitivities may cause allergic reactions when these foods are eventually added to the diet in the latter part of the first year. The age of onset of eczema is considerably earlier than that of asthma and, in many instances, it is the forerunner of asthma.

After early infancy, asthma becomes the prevailing allergic syndrome, and wherever there is a multiplicity of syndromes, asthma is generally one of the complicating conditions. Asthma was also the dominant form in the allergic antecedents of the children studied. It appears, therefore, that the lung structure is phylogenetically predisposed to sensitization in the human species as it is in the guinea pig.

Hay fever is not of great significance in early infancy in the middle Atlantic States. It comes to the fore in the latter part of the first decade and becomes a prominent syndrome in the second decade of life.

Urticaria at all times remains sporadic and shows no particular age alignment.

Though food sensitivities play a very important role in the allergy of childhood, inhalants and contactants play an equally important one. Throughout infancy and childhood reactions to foods and inhalants and contactants run a parallel course. There are many infants and children who react to pollens who do not suffer from hay fever. The pollens may be contributing factors in the eczema or asthma, or they may indicate potential sensitivities which eventually cause the onset of hay fever.

The preponderance of multiple reactivities would suggest that specific therapeutic measures may fail if all the offending factors are not taken into account.

The onset of allergy can in a measure be prevented through control of the diet of the pregnant woman and of the young infant. Cognizance of the important role that environmental inhalant substances play in earliest infancy must lead to prophylactic measures.

The progress of allergy can be interrupted by early diagnosis of the interrelated syndromes and reduction of contact with inciting substances.

It is true that in the present state of our knowledge it is difficult actively to engage the interest of the physician who is unprepared in training and equipment in this subject, which appears to be complicated by so many factors and which from many reports seems to yield but few satisfactory results.

The time must soon arrive, however, when the salient facts, gleaned from the maze of work that is being carried on, will be separated from the chaff and formulated into broad principles that can be more readily applied to general everyday practice.

NEOPLASMS, Malignant, of Nasopharynx, Hauser, I. J., and Brownell, D. H. J. A. M. A. **111**: 2467, 1938.

Malignant neoplasms of the nasopharynx often manifest themselves by symptoms which are extranasal in nature.

Cervical swellings, changes in the tympanic membrane, unilateral deafness or stuffy sensation in one ear, pain in the head or throat, diplopia or rectus lateralis paralysis, or unilateral paralysis of any cranial nerves calls for careful examination of the nasopharynx.

All malignant neoplasms arising from the lining epithelium of the nasopharynx appear to be medullary squamous-cell carcinomas. Lympho-epithelioma and transitional-cell carcinoma are believed to be highly undifferentiated forms of squamous-cell carcinoma.

Irradiation is at present the only form of treatment. In spite of poor results, if earlier diagnoses were made, chances of arresting or destroying the neoplasm would be greater.

The nasopharynx should be given careful routine inspection by the otolaryngologist.

POLYCYTHEMIA, Course of, Rosenthal, N., and Bassen, F. A. Arch. Int. Med. **62**: 903, 1938.

Primary polycythemia is a disease of long duration. Occasionally the early stage may be asymptomatic, but usually it is symptomatic.

After a period of years one or more of a variety of clinical conditions apparently develop, as a result either of the hyperactivity of the leucopoietic and megakaryocytic systems or of exhaustion of the erythropoietic system.

In its terminal stages this disease may become leucemic, thrombocythemic, or anemic, or it may reveal various remarkable combinations of any or all of these phases.

SEDIMENTATION RATE, and Non-filament, Filament Ratio in Low Grade Chronic Illness, Stiles, M. H. Arch. Int. Med. **63**: 664, 1939.

Practically all of a group of 292 patients with symptoms of low grade chronic disease showed an increase in the nonfilament-filament ratio, while more than 80 per cent showed an increased sedimentation rate.

The sedimentation rate and the nonfilament-filament ratio showed only minor variations when distributed according to age, sex, or diagnosis. When distributed according to intensity of symptoms, they showed a direct relationship to increases in the severity of illness.

These findings should prove useful in following the course of low grade chronic disease and in evaluating treatment.

PNEUMONIA in Infancy, Sulfapyridine Treatment of, Wilson, A. T. et al. J. A. M. A. **112**: 1435, 1939.

In a study to determine the value of sulfapyridine in the treatment of pneumonia in infants and children, no distinction was made between croupous pneumonia and bronchopneumonia. The authors believe that it is necessary to study a control group

simultaneously with a "sulfapyridine group" and to compare such factors as age, severity of pneumonia, and time at which there is clinical improvement, significant fall in the temperature, and clinical recovery. An analysis of these factors is especially important in a study of pneumonia in early life, since the case fatality rate is so low that it cannot be employed as a criterion for comparison. For example, all the patients in both groups in this study recovered.

Seventy patients with pneumonia, half of whom received sulfapyridine, were observed. Analysis of the characteristics of the disease in the sulfapyridine group and in the control group demonstrated that the two groups were suitable for comparison.

The administration of sulfapyridine apparently shortened the course of the pneumonia by approximately three to four days. By statistical analysis it was demonstrated that the fall in temperature and the clinical recovery were significantly earlier in the sulfapyridine group than in the control group.

The series of cases was too small to allow evaluation of the effect of sulfapyridine in preventing complications of pneumonia.

Two of the patients in the sulfapyridine group manifested a course apparently uninfluenced by the drug; two other patients had a relapse, and one had a series of relapses whenever the use of the drug was discontinued. One patient in the control group had a relapse.

The optimum dosage of sulfapyridine needs further study. Observations indicate that a dosage which secures a level of free sulfapyridine in the blood of approximately 4 mg. per 100 c.c. is therapeutically adequate.

There were marked individual variations in the levels of sulfapyridine in the blood obtained with the doses we employed, namely, from 1 to 1.5 grains (0.06 to 0.1 gm.) per pound of body weight each twenty-four hours. To be sure that an adequate dosage is being maintained, it is necessary to examine the blood frequently, that is, at least daily. The use of a photoelectric colorimeter makes it possible to perform determinations on 0.1 c.c. of capillary blood.

Vomiting and cyanosis were present in about half the patients receiving sulfapyridine. The cyanosis was not sufficient to cause concern in any instance. A number of patients in the control group also manifested cyanosis and vomiting during the acute stage of their pneumonia. Two patients receiving sulfapyridine had cutaneous eruptions. None of the severer drug reactions were encountered.

These preliminary observations on the effect of sulfapyridine on the pneumonias of infancy and childhood and the apparent low toxicity of the drug in the doses employed are sufficiently encouraging to warrant further study.

WASSERMANN REACTION Positive in Spirochetal Infections Other Than Syphilis, Murrell, T. W. Arch. Dermat. & Syph. **39**: 667, 1939.

A case is reported in which an undoubted relationship existed between a positive Wassermann reaction and a spirochetal infection other than syphilis.

It is increasingly evident that the so-called false positive reaction is not a rarity, and if eugenic laws based on serologic reports are to be passed, this fact should be given due consideration. In view of the changing status of such reactions, it may be advisable to abandon the term "false positive" as a part of the syphilologist's nomenclature.

PNEUMONIA, Acute, in Infants and Children in Johannesburg, Kark, S. L., and Jaspan, E. South African J. M. Sc. **3**: 121, 1939.

Seven hundred and eighty-four case records have been examined, of which 548 are those of European children and 236 non-European.

The mortality rate of the non-European children was found to be almost double that of the Europeans. No attempt has been made to discuss the reasons for this difference, as there are far too many essential differences between these groups of peoples which have

not as yet been analyzed. Factors such as nutrition, structure, and mode of living have not been investigated, and it would, therefore, be idle for us to speculate on the various causes of this difference in mortality rate.

A clinical differentiation between the lobar and bronchopneumonic forms of acute pneumonia has been attempted. In both the European and non-European peoples, bronchopneumonia is found to be more common in infancy, whereas in older children the lobar type is more common. Bronchopneumonia appears to be more common in older non-European children than in Europeans. The difference in this series is possibly not significant, but the authors have stressed this aspect of the problem in the hope that other workers will be interested in what might prove to be a very valuable study.

In both groups of peoples the authors have found bronchopneumonia to be a greater cause of death than lobar pneumonia, lobar pneumonia in the European group being a relatively rare cause of death (2.7 per cent). In the non-European group lobar pneumonia caused more deaths, having a mortality rate seven times as high as the European. The non-European mortality rate for bronchopneumonia (50.6 per cent) was 11.3 per cent higher than the European.

The incidence and mortality were found to be highest in infancy in the non-European series as well as the European. At all ages the mortality rate of the non-European was higher than the European.

A slight preponderance of males was noted in the series. There was no significant difference in mortality in the two sexes.

The highest incidence of both lobar and bronchopneumonia was found to occur in the winter months. Variation of mean maximum and mean minimum temperatures from month to month did not appear to have any significant effect on the seasonal incidence.

LEUKEMIA, Phagocytic Activity in, Hirschberg, N. Am. J. M. Sc. **197**: 706, 1939.

Phagocytic studies, sedimentation rates, and bacteriologic studies were made in 20 patients with various types of leucemia.

The number of mature neutrophiles in the peripheral blood of leucemic individuals which showed phagocytic activity was greatly decreased from normal, and the phagocytic activity of the existing cells was also greatly decreased.

The sedimentation rate was increased in most of the acute cases but was normal in most of the chronic cases of leucemia.

Significant bacteriologic findings were noted in most of the acute cases.

It seems probable that leucemia is not itself an infectious disease but that infection so often found associated with this disease is superimposed upon the leucemia. This may be due to the decreased resistance of the leucemic individual, as evidenced by the decrease in phagocytic activity of the mature neutrophiles.

MENINGITIS, Experimental Type II Pneumococcic, Chemotherapy of, Gross, P., Cooper, F. B., and Lewis, M. Am. J. M. Sc. **197**: 609, 1939.

A cerebrospinal meningitis has been produced in rats with a type II pneumococcus.

Sulfanilamide therapy of this disease effected a marked reduction in mortality as well as in incidence and severity of the lesions.

Therapy with 4,4'-di-(acetylamino)-diphenylsulfone was less effective against this infection than sulfanilamide.

Sulfanilamide is suggested as an adjuvant to specific serum therapy, or as the primary therapeutic agent when such serum is not available, as a means toward an effectively lowered mortality rate of pneumococcic pneumonia and meningitis in man.

PNEUMONIA, Pneumococcus Type I, Specific Treatment of, Finland, M., and Brown, J. W. Am. J. M. Sc. **197**: 151, 1939.

The death rate among patients with type I pneumococcus pneumonia treated with concentrated type-specific antibody at the Boston City Hospital has been regularly one-half or less that of similar contemporaneous nonserum-treated patients. This has remained true

in recent years in spite of the fact that the proportion of persons treated with serum has increased from 43 to 89 per cent. Of the patients treated with serum before the end of the fourth day, the death rate is less than one-third of the nonserum-treated mortality.

Bacteriemic and nonbacteriemic persons are equally influenced. The greatest reduction in death rate occurs in patients under 50, but those of other age groups are probably also beneficially affected by serum treatment. Empyema occurred after serum treatment, chiefly in bacteriemic patients, and its frequency was proportional to the delay in beginning treatment.

Type I antipneumococcus sera produced in both horses and rabbits were potent and effective. There are insufficient data to indicate any superiority of the one over the other. There were no immediate allergic type of reactions observed in the rabbit serum recipients, but thermal reactions and serum sickness were somewhat more frequent among them.

Sulfanilamide in the small number of cases in which it was used alone or with serum seemed to influence the course of type I pneumococcus pneumonia only slightly, if at all.

BRUCELLOSIS, Significance of Standard Laboratory Procedures in the Diagnosis of, Menefee, E. E., and Poston, M. A. Am. J. M. Sc. **197**: 646, 1939.

There appears to be a direct relationship between raw milk consumption and skin reaction to brucellergin.

Skin tests are not without danger, since severe local and systemic reactions are not infrequent.

There appears to be little correlation between skin reactivity and measurable antibody content of the serum.

Endermic injection of brucellergin will cause a definite increase of circulating antibodies in allergic patients.

Brucellergin followed by typhoid vaccine will cause a greater and more prolonged increase of circulating antibodies than brucellergin alone.

Any febrile reaction will cause an increase of circulating antibodies.

In survey work a better immunologic picture is obtained by measuring the antibodies at the height of the antibody response to brucellergin.

Negative skin tests, agglutinins, and opsonocytophagic reactions do not rule out the possibility of Brucella infection.

Positive skin tests, low agglutinins, and a moderate opsonocytophagic reaction, even when accompanied by suggestive signs and symptoms, do not prove the diagnosis of brucellosis.

SYPHILIS, With Special Reference to the Incidence in Relationship to Age Groups and Status of Therapy at the Date of Infection, Hansmann, G. H. J. A. M. A. **12**: 1796, 1939.

Syphilis was studied in the various age groups in Milwaukee, with special reference to incidence and the consequences of the disease as well as the relationship of the incidence to the development of means for recognition and treatment of the condition.

Dependence was placed on routine admissions, presentation of parents for blood transfusions, and post-mortem examinations for reliable sampling of the public in the study.

The high incidence of syphilis which prevails in the age group who acquired their syphilis before the advent of the discovery of the spirochete, the development of important arsenic and bismuth preparations, and the knowledge of the serologic tests, indicate that the ground work has been laid for an imminent spontaneous fall in the incidence of syphilis.

The incidence of syphilis varies from one locality to another, depending on the alertness of the profession, the cooperation of the patient, and the provisions for the care of the indigent, named in the order of their importance.

There can be no intelligent discussion of syphilis on the basis of the number of serologic tests per thousand of population. The least information for an intelligent discussion would appear to be the number of individuals represented by the tests per given area, the personal and economic consequences of the disease, the contribution therapy makes to the individual, the functional and anatomic integrity of the body resulting from causes other than syphilis, and the inadvertent serious consequences of the treatment.

In all but one of the post-mortem examinations made, the morbid anatomy of syphilis was at an irreducible residuum.

LYMPHOGRANULOMA VENEREUM, Clinical Test of a New Method to Determine the Presence of the Virus of, in the Differential Diagnosis of Intestinal Involvements, Paulson, M. J. A. M. A. 112: 1788, 1939.

The experimental and clinical evidence to date supports the view that the virus of lymphogranuloma venereum in intestinal discharge or tissue is of pathogenic significance. The only direct clinical method now available to determine the presence of the virus in such sources is in the use of bowel antigen. When bowel antigen is positive, it indicates the virus of lymphogranuloma venereum in the bowel tissue or discharge from which it is obtained.

Bowel antigen is important because:

1. The clinical picture is not pathognomonic. The so-called typical state of intestinal lymphogranuloma venereum is not always caused by this virus.

2. Some cases clinically indistinguishable from nonspecific ulcerative proctitis or colitis are found associated with the virus of lymphogranuloma venereum by means of bowel antigen.

3. The pathologic picture, grossly and histologically, is not pathognomonic. Bowel antigen may aid in differential diagnosis ante and post mortem.

4. The Frei antigen alone in differential diagnosis is inadequate because:

(a) The patient may give no reaction to Frei antigen, and bowel antigen may indicate virus presence (anergy).

(b) A positive Frei reaction may be produced by an antedated or healed virus infection, or one unrelated to the bowel disturbance.

PNEUMOCOCCI, Use of Serum Tellurite Agar for the Isolation of, Petran, E. J. A. M. A. 112: 1688, 1939.

Practically all strains of pneumococci produce very characteristic colonies on tellurite agar. The colonies, which vary in size from 0.1 to 1 mm. in diameter, are very thin and flat, entire and gray with a brownish gray center. Some give the appearance of having a raised edge and a depressed center. Recently isolated and virulent type III pneumococci produced large (from 3 to 4 mm.) raised, watery, mucoid, light gray colonies. While the morphologic appearance of the colonies of pneumococci on this medium is very much like that produced on ordinary blood agar, the pneumococcus colonies are easily distinguished from those of streptococci. The colonies are not always typical, however, until the plates have been incubated for twenty-four hours.

In general, streptococci produce raised, black colonies on serum tellurite agar. Certain strains of hemolytic streptococci which produce large mucoid colonies are difficult to distinguish from the colonies of type III pneumococci. The mucoid streptococcus colonies are, however, darker than pneumococcus colonies and do not usually have an entire edge. The tellurite medium has the added advantage that proteuslike and spore-forming bacteria do not produce spreading colonies.

REVIEWS

Alcohol in Moderation and Excess*

THIS is undoubtedly the best discussion of the subject that has appeared since Emerson's *Alcohol and Man* was published several years ago. It has the advantage of brevity. Written at the request of the Virginia State Legislature for school use, it aroused such antagonism among the prohibitionist forces, that it was finally burned, not in effigy but in fact, in the furnace of the state capitol, the building designed by Thomas Jefferson, lover of freedom of speech. The one thousand copies ordered by the legislature were so destroyed, before they were even read by the legislators.

This book may be most highly recommended as an unbiased discussion of the pros and cons of the subject.

Surgical Pathology of the Diseases of the Mouth and Jaws†

THIS is the tenth of a series of monographs by Professor Hertzler and, like its predecessors, may be expected to take its place as an outstanding reference text. He writes with a vigorous and refreshing style upon the lessons drawn from his ample experience. It is to be regretted that, as the author states in his preface, this is the last of the series.

His viewpoint is broad and it is apparent that his experience in tissue pathology is as extensive as his experience in surgery. What he has to say is well worth reading. The book is excellently printed and the illustrations are on a par with the preceding volumes of the series. The photography and microphotography are of outstanding excellence and are well reproduced.

This volume is well worth its price and can be highly recommended.

Pocket Medical Dictionary‡

SO MANY, so rapid, and so inevitable are the changes in medical terminology occurring almost from day to day that a medical dictionary is an essential reference.

This pocket edition, while, of course, not replacing the large and more complete volumes, is still a multum in parvo, containing over 40,000 words and many useful tables.

As a convenient and handy reference it can be well recommended. Any book which has gone through seventy-five printings has established itself as a standard text.

A B C of the Vitamins§

WHILE it is possible that this book is intended primarily for the laity, the physician may profitably add it to his reference library.

Despite the voluminous data, not altogether free from complexity, which has accumulated concerning the vitamins, Miss Gregory, by means of cleverly constructed charts, presents their story not only clearly, but in a most interesting manner. Her charts are accurate in their data and present the material in a clear and concentrated way. This book may be enthusiastically commended.

*Alcohol in Moderation and Excess. A Study of the Effects of the Use of Alcohol on the Human System. By J. A. Waddell, M.D., Professor of Pharmacology, Materia Medica, and Toxicology, Medical Department, University of Virginia; and H. B. Haag, M.D., Professor of Pharmacology, Medical College of Virginia. Bound in boards and cloth, 184 pages, 25 illustrations, $1.00. William Byrd Press, Inc., Richmond, Va., 1939.

†Surgical Pathology of the Diseases of the Mouth and Jaws. By Arthur E. Hertzler, M.D., Professor of Surgery, University of Kansas. Cloth, 248 pages, 206 illustrations, $5.00. J. B. Lippincott Company, Philadelphia, Pa.

‡Pocket Pronouncing Medical Dictionary. By George M. Gould, A.M., M.D. Revised by C. V. Brownlow. Leatherette, ed. 11, $2.50. P. Blakiston's Son & Co., Philadelphia, Pa.

§A B C of the Vitamins. A Survey in Charts. By Jennie Gregory, M.S. With a Foreword by Walter H. Eddy, Professor of Physiological Chemistry, Columbia University. Cloth, 93 pages, $3.00. Williams & Wilkins Co., Baltimore, Md.

Recent Advances in Chemotherapy*

PERHAPS the most interesting of the newer methods in chemotherapy is the application of sulfanilamide and its derivatives in the treatment of bacterial infections. This new edition, therefore, devotes much space to a review and summary of the pertinent literature.

The value of such a book as this is that under one cover it presents the consensus of investigations from all over the world which originally appeared in specialized and not always readily accessible sources. The physician is thus easily enabled to familiarize himself with the latest advances in chemotherapy and has the further advantage that they are evaluated by competent authority. This is an excellent source and reference book which can be included to advantage in any medical library. A comprehensive author and subject index add greatly to the usefulness of the book.

This book can be recommended as worth while.

Glaister's Medical Jurisprudence and Toxicology†

THIS book needs no introduction for Glaister's Medical Jurisprudence has long been a standard reference work of outstanding reputation.

The present edition shows numerous changes and additions, among the latter notably an account of the procedures followed in the Ruxton case.

As before, this book presents in a distinctive and altogether excellent manner a comprehensive account of medicolegal procedures embodying all the important recent studies. Well written, based upon extensive experience and a thorough familiarity with the literature, this edition, like its predecessors will again take place as an outstanding reference work in this field. While it may well be added to the library of the physician at large, the qualifications of the editor, amply reflected in the book, make this volume of particular interest and value to the pathologist, the lawyer, those associated with "crime laboratories," and, indeed, to all who may come in contact with this field.

Glaister may be recommended without reserve.

The Harvey Lectures‡

THIS, the thirty-third series of The Harvey Lectures, presents eight lectures on varied subjects: The Nature of the Visual Process (Professor Hecht, Columbia); The Pasteur-Meyerhoff Reaction in Muscle Metabolism (Professor Lundsgaard, University of Copenhagen); The Functional Significance of the Lymphatic System (Professor Drinker, Harvard); Transfer of Water and Solutes in the Body (Professor Peters, Yale); Studies on the Cortical Representation of Somatic Sensibility (Professor Bard, Johns Hopkins); The Isolation and Properties of Tobacco Mosaic and Other Virus Proteins (Dr. W. M. Stanby, Rockefeller Institute); The Chemistry and Biology of Male Sex Hormones (Professor Koch, University of Chicago); and Experimental Hypertension Induced by Renal Ischemia (Professor Goldblatt, Western Reserve University).

As usual these are thoughtful papers well deserving study

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*Recent Advances in Chemotherapy. By G. M. Findlay, C.B.E.M.D., D.Sc., Wellcome Bureau of Scientific Research, London. With a Foreword by C. M. Wenyon, C.M.G., F.R.S., Director-in-Chief, Wellcome Bureau of Scientific Research, London. Cloth, ed. 2, 523 pages, $5.00. P. Blakiston's Son & Co., Philadelphia, Pa.

†Glaister's Medical Jurisprudence and Toxicology. Edited by John Glaister, M.D., D.Sc., Barrister-at-Law; Regius Professor of Forensic Medicine, University of Glasgow; Formerly Professor of Forensic Medicine, University of Egypt and Medicolegal Consultant to the Egyptian Government. Cloth, ed. 6, 747 pages, 107 illustrations, 8 plates, $8.00. William Wood & Co., Baltimore, Md.

‡The Harvey Lectures, delivered under the auspices of the Harvey Society of New York, 1937-1938. Series XXXIII. Cloth, 275 pages, 52 figures, 1 colored plate, $4.00. Williams & Wilkins Co., Baltimore, Md.

The Patient Is the Unit of Practice*

THIS is a book which every physician, particularly every young physician, can read with profit to himself and especially with profit to his patients. In an eminently readable and interesting way, it is devoted to a well-balanced discussion of an age-old, yet often overlooked, principle, to wit: that the patient must be regarded as an individual rather than the embodiment of a symptom or disease.

Dr. Propst takes as his text a quotation from Sydenham: "More importance is to be attached to the desires of the patient, provided they are not excessive or dangerous, than to doubtful and fallacious rules of medical art."

This is the voice of wisdom tempered by experience. It is true that medicine should be scientific. But it is true that it is very easy to become, without realizing it, pseudo-scientific; to bury the patient as an individual under a lengthy and detailed history which takes note of everything, except the fact that he is a person, an individual with individual traits which color and influence his reaction to stimuli of whatever nature; to lose sight of him as a person in a snowstorm of reports and radiographs and cardiograms and what not; in a word, to be scientific ad nauseam and utterly fail as a ministering physician.

Dr. Propst divides his book into three main sections: I. The Nature of Disease; II. The Diagnostic or Working Hypothesis; and III. Therapeutic Principles.

This book abounds in common sense and radiates practical experience. It can be read and reread. It should be read once, at least, by everyone engaged in the practice of medicine. This is a "must" item for the physician's library.

Recent Advances in Forensic Medicine†

THE current vogue of "whodunit" fiction has made it common knowledge that there is a close relationship between medicine in its varied phases and the study of legal problems.

In this book Smith and Glaister review the recent advances in forensic medicine and present in a succinct and clear-cut manner much that otherwise could be found only in specialized and not always easily accessible sources.

The first four chapters of the book are devoted to the medicolegal problems related to injuries from projectiles and cover the subject in a thorough and excellent manner. There is an excellent chapter, abundantly illustrated, on the identification of hairs; a discussion of the medicolegal applications of precipitin tests; an epitome of the newer studies related to blood grouping, including the M and N factors; a discussion of the quantitative determination of carbon monoxide in the blood, of spectroscopy and the use of ultraviolet and infrared rays in medicolegal investigations of larval infestation and putrefaction, and of the concentration of alcohol in the blood and its relation to intoxication, and an appendix concerning the demonstration of powder residues on fabrics.

The authors are well-known medicolegal experts and write from an extensive experience.

This book is of value to the specialist in forensic medicine and to the lawyer engaged in this field; to the pathologist within whose purview such problems may fall, and to the physician who must have at least a working knowledge of their extent and variety.

While it is not the purpose of the book to make its reader a medicolegal expert, it is a definitely useful and accurately informative book of great interest.

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*The Patient Is the Unit of Practice. By Duane W. Propst, A.B., B.S., M.D., Assistant Professor of Medicine, University of Illinois. Cloth, 219 pages, 4 plates, $3.50. Charles C. Thomas, Springfield, Ill.

†Recent Advances in Forensic Medicine. By Sydney Smith, M.D., F.R.C.P., D.P.H., Regius Professor of Forensic Medicine, University of Edinburgh; and John Glaister, M.D., D.Sc., J.P., Regius Professor of Forensic Medicine, University of Edinburgh. Cloth, ed. 2, 264 pages, 85 illustrations, $4.50. P. Blakiston's Son & Co., Philadelphia, Pa.

Statistical Methods for Research Workers*

THIS new edition of a well-known and deservedly popular book contains many changes and additions so that the book should be even more useful than before.

The relation of mathematics to the presentation and statistical analysis of research data is well recognized and even obvious. Not all engaged in research are sufficiently oriented in the methods of the statistician to apply them to their problems. To these, this book will be of inestimable value for it is clear, understandable, and practical.

Drug Addicts Are Human Beings†

THIS is a book concerned with the narcotic problem.

Dr. Williams believes that the drug addict should be entitled to a freely available maintenance supply of the drug in question; that the Harrison Act is ineffective, works injustice to both physician and addict, that it places both in the power of the unscrupulous narcotic agent, and that, as a consequence, it has given way to a "billion dollar drug racket."

There is no question but that the handling of the narcotic problem leaves much to be desired. But this presentation of it and how it may best be solved would be much more effective if the style of this book were less reminiscent of De Kruif in his best and most hysterical days.

Physical Diagnosis‡

PERHAPS few books on physical diagnosis have been more deservedly popular than Dr. Cabot's for few books of this character have been based on as comprehensive an experience or have presented the subject in a more practical and usable manner.

Those familiar with former editions—and their names are legion—will remember that Dr. Cabot's book was, in essence, a mirror of his own personal experience. Its aim was to express, as stated in the first preface, not merely what had been recommended, but rather what he, by actual experience, had found valuable.

This twelfth edition marks a departure in this respect, but not in the purpose or the character of the book as a whole. Realizing that no one individual experience, no matter how extensive, can embrace all that the modern study of physical diagnosis now comprises, Dr. Cabot has associated with him in the preparation of this new edition Dr. F. Dennette Adams as co-author.

This does not mean that "the personal equation" so characteristic of former editions is lost. On the contrary, it now presents the results of the personal experience, not only of the authors, but also of their colleagues in the Massachusetts General Hospital. The new edition, therefore, embraces even a wider field than its predecessors. It is larger in size and contains numerous entirely new illustrations.

The intensely practical character of the book is evident at a glance just as it is also evident that it represents practical and personal experience. It is without doubt a book of individuality, well organized, well written, well illustrated, and well indexed.

This book can be highly recommended without reserve.

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*Statistical Methods for Research Workers. By R. A. Fisher, Sc.D., F.R.S. Cloth, ed. 7, 356 pages, $3.50. Oliver & Boyd, Edinburgh and London.

†Drug Addicts Are Human Beings. By Henry Smith Williams, M.D., B.Sc., LL.D. Cloth, 273 pages, $2.50. Shaw Publishing Co., Washington, D. C.

‡Physical Diagnosis. By Richard C. Cabot, M.D., Professor of Clinical Medicine Emeritus; and F. Dennette Adams, M.D., Instructor in Medicine, Harvard Medical School Courses for Graduates. Cloth, ed. 12, 846 pages, 309 illustrations, $5.00. William Wood & Co., Baltimore, Md.